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A CYTOGENETIC STUDY OF A CHROMOSOME FRAGMENT IN MAIZE[1]

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INTRODUCTION

EACH of the ten groups of linked genes in maize has been associated with a specific member of the haploid set of ten chromosomes. The morphology of the ten chromsomes has been studied at pachytene by McCLINTOCK (1933) and each member found to differ from the others in such characters as length, position of the spindle fiber insertion region and presence of deeply staining knobs in definite positions, so that they can be recognized by their morphological characteristics.

The fifth longest chromosome of the haploid set of maize has a spindle fiber insertion region that is nearly median, dividing the chromosome into two arms. The ratio of the length of the two arms is 1.1:1.0. In certain strains the longer of the two arms, however, is differentiated at pachytene by the presence of a deeply staining knob that makes it easy to distinguish between the two nearly equal arms of the chromosome.

Plants which possess an extra chromosome V are said to be trisomic for this chromosome. These trisomic plants differ markedly in appearance from their disomic sibs and it is possible to classify a segregating progeny into trisomic and disomic types with considerable accuracy. For this reason plants trisomic for chromosome V have been successfully utilized in an attack on certain problems.

There are two secondary trisomes possible for each primary trisome since the extra chromosome in the secondary trisome is composed of two identical arms, that is, one of the arms of the chromosome is represented twice in the extra chromosome. One of the two secondary trisomes for chromosome V has been found (RHOADES 1933b). The extra chromosome consisted of two short arms of chromosome V. This secondary differed strikingly in appearance from both primary trisomes and disomes.

OCCURRENCE OF A FRAGMENT PLANT

An individual which was intermediate in its appearance between its trisomic and disomic sibs arose in the progeny of a chromosome V primary

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trisome. Cytological examination of this exceptional plant showed that it contained a fragment of chromosome V. Studies at pachytene showed that this fragment consisted of the entire short arm of chromosome V with a terminal spindle fiber insertion region. That the break which produced the fragment occurred exactly at the spindle fiber region is indicated by the fact that in many clear pachytene figures no evidence of any chromatic material on one side of the insertion region was ever found. The nature of this fragment in its relation to a normal chromosome V is shown diagrammatically in figure 1.

FIGURE 1.—Diagrammatic representations of normal chromosome V showing relative length of the two arms, position of spindle fiber insertion region, location of knob and of fragment chromosome which consists of the entire short arm of chromosome V and which has a terminal spindle fiber insertion region. The insertion regions are designated by the stippled areas.

FIGURE 2.—Trivalent groups of the two normal and the fragment chromosomes at metaphase I showing the orientation of the fragment chromosome to be such that it disjoins to the same pole as one of the normal chromosomes.

As previously reported, maize plants trisomic for chromosome V differ markedly in their appearance from their disomic sibs. Especially noticeable are the thick broad leaves with relatively blunt tips, the stubby tassel and the reduced height of the trisomic plants. Plants carrying the short arm of chromosome V in excess are intermediate in appearance between primary trisomes and disomes.

CYTOGENETICS OF FRAGMENT CHROMOSOME

Cytological observations of microsporocytes at metaphase I showed that the fragment chromosome was associated with the two normal chromosomes V in approximately 53 percent of the cases to form a trivalent group. This frequency of trivalents is considerably lower than the 85–90 percent found in plants trisomic for the whole chromosome V. If assortment of the members of the trivalent group is completely random six gametic types are possible. If these six types are produced with equal frequencies the genetic backcross ratio from a plant carrying two dominant and one recessive alleles (AAa) would be $5A:1a$. (This ratio ignores the effect of chromatid crossing over on genetic ratios.) Random assortment

of the three homologous chromosomes occurs in primary trisomes but the occurrence of univalents and their failure to pass to either pole in anaphase results in a deviation of the genetic ratio from a 5:1 to about 3.35:1 (RHOADES 1933a) for genes in chromosome V.

Typical trivalent figures involving the fragment chromosome are shown in figure 2. The orientation on the metaphase plate of the trivalent group was such that the fragment would pass to the same pole as one of the normal chromosomes and was rarely of the type that would permit the two normal chromosomes to pass together to one pole while the fragment went to the other pole. Only four gametic types are usually formed. The theoretical gametic ratio for genes located in the short arm would be $3A:1a$ if the four gametic types are formed with equal frequencies and if the dominant allele is carried in the fragment chromosome and in one of the two normal chromosomes.

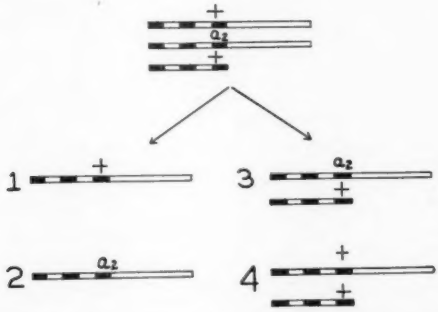

FIGURE 3.—Diagrammatic representation of the four gametic types produced by a fragment plant. The locus a_2, which is included in the fragment, is used to mark the different chromosomes.

The rare occurrence of primary trisomes in the progeny from fragment plants substantiates this idea of a non-random segregation of the members of the trivalent group.[3] Since the spindle fiber insertion region of the fragment chromosome is comparable in size with that of the two normal chromosomes, the failure of occurrence of random segregation indicates that the insertion region does not of itself control chromosome segregation. Such a conclusion has been reached on genetic grounds by DOBZHANSKY and STURTEVANT (1931) in their studies on Drosophila translocations. The four gametic types from a fragment plant are shown diagrammatically in figure 3.

[3] Actually 1.5 percent of the progeny from a fragment plant, used as the female parent, are primary trisomes. The occurrence of these primary trisomes has a slight effect upon the genetic ratios but this has been disregarded to facilitate calculations.

When a trivalent group was present at metaphase, disjunction occurred and the three chromosomes were distributed between the two poles. If a bivalent and a univalent were present, however, the behavior of the univalent was variable. Often it never reached the metaphase plate and remained in the cytoplasm during the formation of the daughter nuclei. If it reached the metaphase plate it was often late in beginning its migration to one of the two poles and consequently was not included in the telophase nuclei. Occasionally the univalent apparently succeeded in reaching one of the poles and was, therefore, included in the nucleus.[4] So far as observed the univalent never divided in the first meiotic division. If a univalent was present it was always the fragment chromosome.

Knowing that only four gametic types are formed, that trivalent groups are formed at metaphase in about 50 percent of the cells, we can calculate the gametic ratio for a gene located in the short arm of chromosome V if we assume that only those fragment chromosomes which were members of trivalent groups reach the pole. Based on the above the expected backcross ratio from fragment plants of AAa constitution with a in one of the normal chromosomes is $5A:3a$ or 37.5 percent recessives.

Studies at pachytene showed that the fragment was often paired with the two normal chromosomes. The fragment was paired in approximately one-half of the microsporocytes. The difficulty in interpreting all figures makes this ratio of doubtful value but there was at least rough agreement between the frequency of trivalent complexes observed at pachytene and at metaphase.

That the insertion region is not the place where synapsis must be initiated is indicated by the following observations at pachytene. In those microsporocytes in which the fragment had paired with the short arms of the two normal chromosomes V the terminal insertion region of the fragment chromosome was usually some distance removed from the insertion regions of the normal chromosome. In most synaptic configurations it was some distance from the terminal insertion region of the fragment to the place where the fragment first paired with one of the normal chromosomes. Pairing between the three homologous arms was always between two of the three chromsomes, that is, one of the chromosomes was always unpaired at any given region. There was never any tendency for all three chromosomes to be associated at the same point. The fact that the insertion region of the fragment chromosome had no tendency to be associated

[4] If the fragment chromosome was paired with the two normal chromosomes at metaphase it had a characteristic V-shaped appearance during anaphase I as it passed to one of the two poles. On the other hand if the fragment chromosome was unpaired at metaphase it had a ball-like or spherical shape at anaphase. In an occasional figure a spherical fragment chromosome accidentally lay so near one of the poles that it probably would be included in the telophase nucleus.

with the insertion regions of the normal chromosomes V indicates that the insertion regions in themselves have no specific attraction. In disomes at pachytene the insertion regions of two synapsed chromosomes lie side by side. This is probably caused by their being brought together by the synapsing of the homologous loci on either side and not because of any specific attraction to one another. It was not uncommonly observed that even though the three homologous arms were involved in a synaptic complex the terminal insertion region of the fragment was attached or stuck to the insertion regions of other pairs of chromosomes. This "stickiness" of insertion regions has often been observed in normal preparations and is not a characteristic feature of the insertion region of the fragment chromosome.

FIGURE 4.—Camera lucida sketch of synaptic relationships of the fragment chromosome and the two normal chromosomes V at pachytene. In this figure the insertion region of the fragment is in juxtaposition with the two insertion regions of the normal chromosomes. One of the normal chromosomes has a deeply staining knob in the long arm.

In those sporocytes in which the fragment was present as a univalent at pachytene it was less contracted than the paired short arms of the two normal chromosomes V with which it is homologous. HUSKINS and HEARNE (1933) observed a similar attenuation in unpaired chromosomes. Non-homologous pairings (foldbacks) were of infrequent occurrence in the unpaired fragment. The equational split of the unpaired fragment chromosome into two chromatids was clearly evident at pachytene. Of considerably more interest was the observation that the terminal insertion

region of the unpaired fragment in some cells, where it was not stuck to other insertion regions, appeared to be split or divided at pachytene. The distal end of the insertion region was cleft to form a heart-shaped structure. As the diagrammatic sketch of the fragment chromosome in figure 5 illustrates there is a concentration of deeply staining chromatin near the insertion region while the more distal portions contain much less deeply staining material. A considerable amount of genetic data strongly suggests that the disjunction of the insertion regions is always reductional in the first meiotic division. DARLINGTON (1931) in his theory of meiosis accounts for this by assuming that the split of the insertion region occurs some time after the equational split of the chromosome into two chromatids. If the observation of the split nature of the terminal insertion region of the unpaired fragment is to be considered applicable to paired chromosomes, then the previous failure to observe the double nature of the insertion region at midprophase is probably due to its homogenous appearance and because its usual interstitial location is such as to conceal the split if it occurred. Only when the insertion region is favorably located, that is, terminally, could the split be evident. There is, of course, the possibility that the split condition of the insertion region of the unpaired fragment is not representative of conditions in paired chromosomes with internal insertion regions. Moreover, if the insertion does split at the same time as the chromosome there is no reason why the two daughter insertion regions cannot behave as "one effective insertion region" at metaphase and anaphase.

LEWITSKY (1931) and others hold that no chromosome has a strictly terminal insertion region. It is true that none of the normal complement of maize chromosomes (MCCLINTOCK 1933) has a terminal insertion region but LEWITSKY'S statement does not hold for the fragment chromosomes described in this paper since conditions for cytological observations are exceptionally good at pachytene in maize and the fragment chromosome clearly had a terminal insertion region. The fragment chromosome was as stable as the regular complement of 20 chromosomes in its behavior throughout all the somatic mitoses and no irregularities were manifested.

LOCATION OF GENES IN LONG AND SHORT ARMS OF CHROMOSOME V

Since the fragment consists of the entire short arm of chromosome V it can be employed in determining which of the genes located in this chromosome are situated in the short arm and which are in the long arm. The A_2 locus will be used as an illustration of how this was accomplished. A plant carrying the fragment and homozygous for A_2 was pollinated by a plant homozygous for a_2 and for A_1CR, the other factors concerned with aleurone color. The F_1 fragment plants were then backcrossed with recessive a_2 pollen. If the A_2 locus is in the short arm of chromosome V a deviation from

a 1:1 backcross ratio will be had for A_2:a_2. The magnitude of the deviation will depend upon the frequency with which the fragment chromosome, which carried the dominant allele, is included in functioning female gametes. The fragment chromosome was paired with the two normal chromosomes at metaphase I in about 50 percent of the microsporocytes. If we assume that a similar condition holds for the megasporocytes and if we make the further assumption that the unpaired fragment chromosome does not succeed in reaching either of the two poles in anaphase I, and is consequently lost, the expected ratio of A_2:a_2 seeds in the backcross of fragment plants is $5A_2$:$3a_2$ seeds. Table I gives the ratio of A_2:a_2 seeds actually found and indicates that A_2 is in the short arm of chromosome V. Disomic sister plants gave 869 A_2 seeds to 890 a_2 seeds or a 1:1 ratio. The average of

FIGURE 5.—Diagrammatic sketch of an unpaired fragment chromosome at pachytene. The equational split into two chromatids has occurred and the heart-shaped appearance of the terminal insertion region suggests that it also is double at this stage. There is more deeply staining chromatin near the insertion region than in the more distal portions of the chromosome.

33.6 percent recessives suggests that either approximately 67 percent of the megasporocytes had a trivalent group or else that the unpaired fragment reached one or the other of the two poles in the first anaphase with a considerable frequency. A more useful and efficient test for the presence of any locus in the short arm is the occurrence of fragment plants which are homozygous for the recessive gene in the backcross progeny of a fragment plant carrying one recessive allele in a normal chromosome. If the locus under consideration is in the short arm, no fragment plants will occur, barring the rare occurrence of exceptional individuals through chromatid crossing over, which are homozygous for the recessive allele. This latter test was used for all the genes tested, as it required fewer plants and was of a more critical nature.

Linkage group 5 contains 23 genes. The order of the following genes in the genetic map is correct, $\frac{a_2\ bm_1\ bv\ pr\ ys\ v_2}{0\ 6\ 12\ 31\ 40\ 72}$, according to the data summarized by EMERSON et al (1935). The exact location of the other genes in the linkage map is uncertain. Eight of the 23 genes in this chromosome were tested against the fragment. The V_2, Ys_1, Pr, V_{12}, V_3 and Bl_1 loci were

found to be in the long arm of chromosome V while only A_2 and Bm_1 were situated in the short arm. Of especial interest is the placing of Bm_1 and Bt_1 on opposite sides of the spindle fiber insertion region as these two loci show little crossing over (BURNHAM and RHOADES in EMERSON et al 1935). A_2 is only some 6–7 crossover units from Bt_1, which is in the long arm, and it occupies, at present, the leftmost position of all the factors in the genetic map of chromosome V. If the magnitude of crossing over per unit of physical length is the same in the two arms of the chromosome, and we have no reason to believe otherwise, the length of the genetic map for

TABLE 1

Ratio of A_2:a_2 seed from backcross of $\frac{\frac{a_2}{A_2}}{\underline{A_2}} \times \frac{a_2}{\underline{a_2}}$

PEDIGREE OF FRAGMENT PLANT	A_2 SEEDS	a_2 SEEDS	PERCENT RECESSIVES
2524 6	187	87	31.8
2524 14	166	66	28.4
2525 9	206	118	36.4
2525 6	228	130	36.3
2525 3	59	25	29.8
2524 25	174	77	30.7
2525 2	117	66	36.1
2525 8	92	44	32.4
2524 29	97	47	32.6
3117 a	195	111	36.3
Total	**1521**	**771**	Aver.%=**33.6**

chromosome V is due for a considerable extension as new genes are located in the short arm to the left of A_2. Three-point backcross data for Bm_1-Pr-V_2 are available. Bm_1 is so close to the insertion region that we can consider the amount of crossing over between Bm_1-Pr essentially equal to that between the spindle fiber and Pr. Let us calculate, as best we can, the approximate length of the genetic map for the long arm of chromosome V. The three-point backcross data give the following recombination values: $Bm_1-Pr=22.3$ percent, $Pr-V_2=43.4$ percent. According to HALDANE'S (1919) table for estimating the map distance from recombination percentages the map distance for the Bm_1-Pr interval is 24.5 units and that for the $Pr-V_2$ interval is 60.8 units. The sum 85.3 units gives an estimate of the map distance from the insertion region to the V_2 locus but we do not know how far removed V_2 is from the end of the long arm. Since the two arms of chromosome V are nearly equal it appears that the length of the genetic map for this chromosome will exceed 170 map units.

In the course of the studies with the fragment several plants were obtained which carried either recessive a_2 or bm_1 in the two normal chromosomes V and the dominant allele in the fragment chromosome. Considering the bm_1 locus these plants were of the following constitution: $bm_1Pr/bm_1pr/Bm_1$ and for the a_2 locus had $a_2pr/a_2pr/A_2$ constitution. When a plant carrying the bm_1 gene was used in reciprocal crosses with a recessive individual the following results were obtained:

From the above data where the fragment was used as the pollen parent it is evident that even though the fragment plant was Bm_1 in phenotypic appearance it bred true for bm_1 excepting those cases where fragment pollen functioned or where a crossover had occurred which transferred the Bm_1 allele to a normal chromosome.

The following calculations can be made from the data in table 2. (1) The percentage of functioning eggs carrying the fragment chromosome was 33.

TABLE 2.

FRAGMENT PLANT AS FEMALE	
2N PROGENY	2N+FRAGMENT PROGENY
Pr bm_1—62	*Pr* Bm_1—31
Pr Bm_1—1	*pr* Bm_1—35
pr bm_1—64	*wh* Bm_1—19
wh. bm_1—45	

FRAGMENT PLANT AS MALE	
2N PROGENY	2N+FRAGMENT PROGENY
Pr bm_1—146	*Pr* Bm_1—5
Pr Bm_1—1	*pr* Bm_1—1
pr bm_1—109	*wh* Bm_1—2
pr Bm_1—1	
wh. bm_1—123	

The above crosses were segregating for colored and colorless aleurone. The colorless (wh.) class was included because of the bm_1 data.

(2) The frequency of crossing over between the fragment chromosome and one of the normal chromosomes in the Bm_1-insertion region was .5 percent. (3) Two percent of the functioning pollen from the fragment plant was hyperploid for the fragment chromosome. Incidentally the transference of the Bm_1 gene from the fragment chromosome to one of the normal chromo-

somes could be used as cytological proof of genetic crossing over if such evidence were needed after the conclusive work of STERN (1931) and CREIGHTON and MCCLINTOCK (1931). Obviously a more critical proof would be the transference of the recessive bm_2 allele from a normal chromosome to the fragment chromosome. Such crossovers have occurred in other progenies.

More extensive data from reciprocal crosses similar to the above were obtained for the A_2 locus. The percentage of fragment eggs was 33. The percentage of functioning fragment pollen was 1.2 and the amount of crossing over, based on a thousand individuals, between the fragment chromosome and the normal chromosomes in the A_2-insertion region interval was six percent. JENKIN'S (1934) data places A_2 and Bt_1 7 units apart. Therefore the map distance from A_2 to the insertion region is not more than 7 units. The frequency of crossing over between the fragment and the normal chromosome in the A_2-insertion region interval was six percent. It would seem then that the frequency of crossing over in this interval was the same in disomes as in fragment plants. But the fragment is associated with the normal chromosome in approximately 50 percent of the microsporocytes. Therefore the cross over value of six units should be doubled. This indicates that crossing over for this interval, when the fragment is associated with the two normal chromosomes, occurs considerably more frequently in fragment plants than in diploids.

The results obtained from this study of the fragment chromosome agree with previously known facts concerning the order of genes in this chromosome. MCCLINTOCK (1932) placed the Bm_1 locus in the short arm near the insertion region from her work with ring-shaped fragments. Moreover, cytogenetic studies with a reciprocal translocation (RHOADES 1933c) indicated that Bt_1 and Bm_1 were both near the insertion region of chromosome V. STADLER (1935) obtained a deficiency for the V_3 locus. This deficiency occurred in the long arm of the chromosome. In addition to substantiating the above, the fragment studied places the two closely linked genes Bt_1-Bm_1 (1–2 units apart) on opposite sides of the insertion region. Moreover it definitely places six loci in the long arm and two in the short arm of chromosome V.

When plants of $bm_1Pr/Bm_1pr/Bm_1$ constitution were used as the pollen parent in a backcross the following data were obtained: 515 *Pr* bm_1:568 *pr* Bm_1:383 *Pr* bm_1:386 *pr* bm_1. These data give a recombination value of 41.5 percent for the $Pr-Bm_1$ interval. Diploid sister plants had a crossover value of 22.3 percent for the same region. This large and highly significant increase in the amount of crossing over in the long arm of chromosome V in fragment plants is inexplicable, as somewhat comparable data from Drosophila showed no increase in crossing over in that arm which

was not represented in the duplicating fragment (RHOADES 1931). Unfortunately only the *Pr*-insertion region interval was involved in the present studies. More data on this problem will be obtained later.

SUMMARY

1. A plant which was intermediate in appearance between the trisomic and disomic individuals occurred in the progeny of a plant trisomic for chromosome V. This exceptional plant carried in addition to the normal complement of 20 chromosomes a fragment of chromosome V. The fragment chromosome consisted of the entire short arm of chromosome V with a terminal spindle fiber insertion region.

2. The cytological behavior of this fragment chromosome was studied at pachytene and metaphase in microsporocytes. The fragment chromosome was associated with the two normal chromosomes in approximately one-half the cells. Segregation of the three members of the trivalent groups was not random, but in such a manner that the fragment chromosome usually accompanied one of the normals when it passed to a pole.

3. The spindle fiber insertion region of the fragment chromosome was observed to be split or double in those cells where it was lying free in the nucleus as a univalent. The equational split into two chromatids was clearly evident.

4. The V_2 *Ys Pr* V_{12} V_3 and Bl_1 loci were located in the long arm of chromosome V. The Bm_1 and A_2 loci were located in the short arm of the chromosome.

5. Pollen hyperploid for the fragment chromosome occasionally functioned in competition with haploid pollen.

6. Crossing over in certain regions was apparently more frequent in fragment plants than in diploids.

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FACTORS DETERMINING CONJUGATION IN PARAMECIUM AURELIA

I. THE CYCLICAL FACTOR: THE RECENCY OF NUCLEAR REORGANIZATION

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Received March 9, 1936

INTRODUCTION

IN *Paramecium aurelia* a number of factors interact to determine the occurrence of conjugation. Most attention has in the past been given to the role of environmental conditions. In this paper an internal factor is dealt with: nuclear reorganization of the type occurring both at endomixis and at conjugation. As will be shown, the relation of these nuclear processes to the subsequent occurrence of conjugation is, in the one stock of *P. aurelia* investigated, very different from what is commonly assumed.

MAUPAS (1889) held that conjugation could occur—or, at least, could result in viable exconjugants—only during the period of sexual maturity. This period was assumed to begin long after the last conjugation and to end when the degenerative alterations characteristic of "senescence" began. MAUPAS' conception of sexual maturity was extended to *P. aurelia*, without direct evidence, as a result of his investigations of other species. Indeed, direct study of *P. aurelia* yielded an embarrassing fact which led MAUPAS to remark that under conditions not yet understood, the normal period of "youth" might be abbreviated in certain species. The fact was the existence of two macronuclei in each of the two members of one pair of conjugants (fig. 33, Pl. 13, loc. cit.). He suggested the possibility that these conjugants might still be in the process of reorganization from a previous conjugation. Subsequently, similar but more convincing observations of this kind have been reported in *P. caudatum* (DOFLEIN 1907; KLITZKE 1914), in *P. multimicronucleatum* (MÜLLER 1932), and in other genera. Nevertheless, in one form or another, many workers down to the present time have held that between two successive conjugations a long period of vegetative multiplication must intervene. Thus, HERTWIG (1889 and later papers) maintained that by long exercise of vital functions the volume ratio of the nucleus to cytoplasm was disturbed and required conjugation or an equivalent process to rectify it. According to this view, the disturbed nucleoplasmic ratio led to a high and increasing rate of fission; and the overdriven life activities led to conjugation. SIMPSON (1901) supported MAUPAS in his report of failure to induce conjugation in *P. aurelia*

during the "period of puberty." However, details are lacking and presumably there were no contemporary "mature" controls in which conjugation was induced.

On the other hand, the falsity of this conception of the place of conjugation in the "life cycle" of *P. aurelia* has been clearly demonstrated. JENNINGS (1910) showed for one race that the interval between successive conjugations could regularly be reduced to two or three weeks, sometimes less. In one instance, the interval was reduced to five days and four fissions. Other races, however, could not be induced to yield such short intervals, some conjugating only twice in about two years. JENNINGS held that the minimum interval between conjugations depends upon how much time is required to bring about the three requisite successive stages of starvation, abundant feeding, and decline in multiplication; and that this can be done quickly in some races, but requires a long time in others. Another foundation of the Maupasian sexual maturity conception was completely blasted by WOODRUFF (1908 and later papers). He showed beyond reasonable doubt that under adequate cultural conditions a "life cycle" was nonexistent. At last reports (1932), his stock had been cultivated for 25 years with no indication of a cycle; for eight of these years (5071 fissions) the occurrence of conjugation in the main culture was definitely precluded by means of the daily isolation culture technique. Not only did he show that conjugation was unnecessary, but repeated attempts to induce conjugation were successful only twice: once after more than $6\frac{1}{2}$ years of culture (at about the 4,100th generation) and once after 13 years of culture (after more than 8,000 generations). Further evidence on the periodicity of conjugation was supplied by HOPKINS (1921), who found intervals between successive conjugations as low as 11, 15, 18, 21, 26, 27, and 34 days in some stocks, and as high as three months in other stocks. He reported that the periodicity was very different in different stocks, varying from about three weeks in some to about six weeks in others, and that there was no evidence of periodicity in other stocks. HOPKINS further maintained that the tendency to conjugate was gradually lost under prolonged conditions of rapid growth and could be restored only by a longer or shorter period of dormancy. It appears difficult to reconcile this with WOODRUFF's experience.

In addition to these reports on periodicity in the occurrence of conjugation in *P. aurelia*, there are in the literature a number of scattered observations whose bearing on the question will become clear only after the experiments that follow have been described. From what has already been reviewed, however, it is clear that in *P. aurelia* there is no "life cycle" including a period of "sexual maturity," that the interval between successive conjugations may be as short as five days, and that periodicity in the recurrence of conjugation differs greatly from race to race.

EXPERIMENTAL PROCEDURE

In the present experiments the stock of *P. aurelia* employed (here designated stock R) was derived from a single individual in the spring of 1929. Numerous investigations upon this stock have already been published by investigators in this laboratory. It consists of many biotypes with diverse characteristics. In typical biotypes, conjugation is often readily induced by the following simple procedure: collect the surplus animals from isolation cultures into fresh culture medium in a small dish and keep them at 31°C. This method was standardized as follows.

The culture medium employed, both for the isolation and the mass cultures, consisted of a lettuce infusion inoculated with the bacterium *Flavobacterium brunneum* and the alga *Stichococcus bacillaris*. In preparing this, powdered desiccated lettuce and redistilled water, in the proportion of 1.5 grams of lettuce to a liter, were boiled for five minutes and filtered at once. The filtrate was distributed into pyrex flasks containing an excess of $CaCO_3$ (Kahlbaum). The $CaCO_3$ brings the pH up to 7.2 and narrowly limits its diurnal variation. The flasks were stoppered with cotton and autoclaved. From this stock fluid, the culture medium was prepared daily by adding to 20 cc of the filtered fluid one 1 mm loop of the bacterium from a 3 to 5 day old slant and three 2 mm. loops of the alga from an 18 day old slant.

The daily isolation cultures were carried on pyrex double depression slides containing two drops (approximately 125 mm^3) of culture medium in each depression. Into the medium in each depression a single paramecium was introduced and kept with its progeny for one day at $27.5° \pm 1.0°$C. At the end of 24 hours, a record was made of the number of paramecia in each depression, and one paramecium from each depression was transferred to fresh medium on a fresh slide. Of the remaining animals, one or more were stained with acetocarmine to ascertain the nuclear condition, and the rest were either discarded or put into a mass culture with animals from other isolation cultures.

The mass cultures, consisting of animals from the isolation cultures (usually twelve in number), were set up in shallow dishes in 10 drops of culture medium and kept at $31.0° \pm 1.0°$C. Such cultures were favorable to the occurrence of conjugation and were used for that purpose in all the experiments here reported.

In both the isolation and mass cultures it was necessary, for the purposes of this study, to detect with certainty the occurrence of conjugation; and also of endomixis, since the latter is a factor in the determination of conjugation. This was done as follows:—

Conjugation.—In daily isolation cultures, conjugation rarely occurs. When it does occur, it is only in lines that multiply very rapidly, and then

only near the end of the 24 hour interval between observations. Hence it cannot be missed in the course of daily observations. In the experiments here reported, it never occurred in isolation lines. In mass cultures, on the other hand, observations at 24 hour intervals cannot be depended upon; but observations at 12 hour intervals are reliable. Conjugants remain united about eight hours, and different pairs in a conjugating culture begin conjugating at various times over a period of 12 hours or more. Hence, the rule employed was to observe all mass cultures at intervals not exceeding 12 hours.

Endomixis.—In both isolation and mass cultures, the condition of the macronucleus was the criterion of endomixis. Complete disintegration of the macronucleus (into either ribbons or spherical fragments), or fragments plus the lightly staining anlagen of the new macronuclei, were required at some point in the isolation line and among some individuals of a mass culture before concluding that endomixis had occurred. The finding of fragments associated with a whole macronucleus was not, in itself, considered sufficient evidence of endomixis. Actually, very few cases arose in which doubt was possible, and these few were excluded from the experiments.

In isolation cultures, samples of each line were stained every day; the number of individuals stained in each line was at least equal to the number of fissions in that line during the preceding day. Since on the same day different individuals in the same isolation culture frequently differ in their nuclear condition (for example some may be in endomixis, others not), the nuclear condition found in the stained animals is not necessarily the same as in the animal retained to continue the line; but it does definitely indicate the nuclear condition on the preceding day of the common ancestor of the stained animals and of the animals kept alive to continue the line. Thus, if this common ancestor was not in endomixis, its descendents stained the following day would show either no endomixis or very early stages; if the former was in very early endomixis, then the latter would show middle stages of endomixis; if the former was in the midst of endomixis, then the latter would show late stages; and if the former was in late stages of endomixis, the latter would show no endomixis at all. By correlating the observations made on the same line of descent on successive days, it can be definitely ascertained, in practically all cases, whether endomixis has occurred. The rare cases in which doubt is possible were excluded from the experiment.

In mass cultures, the detection of endomixis requires prior knowledge as to whether conjugation has occurred, for both conjugation and endomixis involve similar macronuclear pictures. If conjugation has not occurred, endomixis may be detected by staining representative samples

from a culture every day until fully fragmented animals are found. Actually, many cultures were not stained every day. It usually could be foretold whether endomixis would appear in any culture, and if so, on what day it would appear. In such cases, staining of samples was often omitted on the preceding days. If conjugation has occurred in a culture, the detection of endomixis involves deciding whether all the fragmented animals found are exconjugants or whether some are endomictic animals. When the proportion of fragmented animals is nearly the same as the proportion of recent conjugants, it is practically impossible to make this decision. On the other hand, when the proportion of fragmented animals is much greater than the proportion of recent conjugants, then it is clear that endomixis is also in progress.

RELATION OF THE OCCURRENCE OF CONJUGATION TO THE RECENCY OF NUCLEAR REORGANIZATION

In usual biotypes of stock R of *P. aurelia*, conjugation occurs only if endomixis or conjugation has recently taken place. This unexpected relation is demonstrated as follows. If two mass cultures are set up at the same time, in the way above described, one containing animals from isolation lines that have recently undergone endomixis or conjugation, the other containing animals from isolation lines that have not recently undergone endomixis or conjugation, it is found that the former promptly conjugate, while the latter do not.

Such experiments were repeated many times through a period of more than a year, and the results were always the same. There were examined 131 cultures containing descendents of animals that had recently undergone endomixis or conjugation, and all of these quickly yielded conjugants. Of cultures containing descendents of animals that had not recently undergone endomixis or conjugation, 232 were examined; none of these yielded conjugants.

Thus, in the period following endomixis or conjugation, the paramecia undergo a change that is reflected in their reaction to the environmental conditions favoring conjugation. During the first part of this period, they react to the standard conditions by conjugating; but in the later part of the period, under the same standard conditions, they fail to react by conjugating.

At what point does the change in reaction occur? Is the change sudden or gradual? To answer these questions, mass cultures were set up on successive days of the period following endomixis or conjugation. This was managed by carrying a group of isolation lines, all of which had been in endomixis or conjugation the same day, and setting up daily a mass culture of the surplus animals from these. (When any of the isolation lines under-

went endomixis again, these were of course dropped from the experiment.) Thus, series of mass cultures were obtained, differing by intervals of one day in the time since they had undergone endomixis or conjugation. Their reaction to the standard conditions favoring conjugation was determined.

In all, there were studied in this way seven series[1] covering the period following endomixis, and one series following a previous conjugation. In three of the series (these were for the period succeeding endomixis), the change in reaction occurred suddenly. Up to a certain time conjugation occurred abundantly in the mass cultures; beyond this time it did not occur at all. In the other five series, there was an intermediate period in which conjugation occurred in only a small proportion of the animals of a culture.

The times at which the change in reaction occurred differed in the different series. The period of abundant conjugation lasted from five days up to twelve days in some series. That is, in some of the series, abundant conjugation occurred only in mass cultures set up on any of the first five days after the climax of endomixis, while in other series it occurred in cultures set up during a longer period after endomixis—up to twelve days in one series.

The period of scanty conjugation also varied. In two series it lasted up to the twelfth day; in one series up to the fourteenth day; in one series up to the sixteenth day. In the series begun with exconjugants it lasted up to the twentieth day. In this period of scanty conjugation only a dozen pairs, or fewer, would be found in any of the densely populated mass cultures. While these few animals were conjugating, many of the non-conjugating individuals were undergoing endomixis.

In the mass cultures set up at longer periods after endomixis or conjugation, conjugation fails to occur, but endomixis occurs in an even higher proportion of the individuals. This proportion increases as the time since the previous endomixis or conjugation increases, until finally it reaches 100 percent. These relations will be set forth in detail in a later paper.

The further history of these late cultures, in which a new endomixis occurred, is of great interest for the relation of the occurrence of conjugation to the occurrence of a previous endomixis. Of such cultures 124 were studied. Some fresh fluid was added to most of them at the time of endomixis. Such fresh fluid did not cause conjugation at the start of these mass cultures, at a period long after endomixis. Of the 124 cultures, conjugation began in every one within three days after the majority of the animals had undergone endomixis.

In many of these cultures it is entirely clear that the conjugating pairs

[1] I am grateful to Dr. Ruth Stocking Lynch for assistance in the study of one of these series.

were the descendents of individuals that had just undergone endomixis. (1) In some cultures, examination shows that endomixis is universal; all the individuals go through it and three days later many of them conjugate. (2) Further, many of the conjugants, if stained in early stages of conjugation, show besides the entire macronucleus (or one just beginning to unravel into ribbons), a number of the pale macronuclear fragments left over from the recently preceding endomixis.

The experiments and observations described above demonstrate that, in this stock of *P. aurelia*, conjugation may readily be induced immediately after the completion of endomixis, though at other times it does not occur or occurs but scantily. This relation is of practical importance in cases in which it is desired to bring about conjugation. It affords a thoroughly dependable method of obtaining abundant conjugation within 24 hours when a large number of recent ex-endomictics are available. When the number of such individuals is as great as 500 to 1,000, conjugation sometimes begins before all have been collected into the mass culture, and hundreds of pairs will be found within 12 hours.

The fact that the similar processes of endomixis and conjugation thus normally occur so close together appears remarkable. A similar close association may exist between two successive conjugations. To test this, 22 mass cultures were set up with conjugants, there being at the beginning in the different cultures from 7 to 53 pairs. All soon yielded a new set of conjugants. The average interval from the original conjugation to the next one was 3.2 days.

To determine whether conjugations can continue to succeed one another at such short intervals, two series of cultures were followed. In each series, when conjugation occurred in any culture, some of the conjugants were removed to a new dish in fresh fluid to start a new culture. In these series, as in all experiments involving conjugants, only those pairs were used that had been observed to remain firmly united for at least three hours. This avoids the danger of using pairs that do not actually complete conjugation. The two series gave similar results. In one, eleven successive conjugations were induced at intervals of 4, 5, 3, 3, 4, 6, 2, 2, 1, and 1 days respectively. In the other series eight successive conjugations were induced at intervals of 2, 3, 2, 3, 2, 3, and 2 days respectively. There appears no reason to doubt that such series of successive conjugations could be continued indefinitely.

DISCUSSION

The results just described show clearly that in stock R of *P. aurelia* conjugation is readily induced during the several days following conjugation or endomixis, but that if a longer time elapses another endomixis must occur before conjugation can be repeated. In other stocks of the same

species these relations do not hold, as will be shown in a later paper. Nevertheless, numerous observations scattered through the literature show that similar relations do hold in at least some other stocks.

It has already been pointed out that the occurrence of very short intervals between successive conjugations was suspected by MAUPAS (1889) and demonstrated by JENNINGS (1910). Similar results have been reported in *P. putrinum* by BÜTSCHLI (1876) and JOUKOWSKY (1898); in *P. caudatum* by DOFLEIN (1907), KLITZKE (1914), and others; in *P. multimicronucleatum* by MÜLLER (1932) and GIESE (1935); and in other genera.

Clear instances of conjugation within several days after endomixis are rarely found in the literature, partly because endomixis was unknown or insufficiently appreciated by workers prior to 1914, and partly because it has been neglected by later workers on conjugation. There is, however, one clear case in the work of WOODRUFF (1914). He reports (p. 237) that the first conjugation in his long cultivated stock occurred on the fifth and sixth days after setting up a mass culture with animals in generation 4,102. Reference to the paper of WOODRUFF and ERDMANN (1914, p. 437) shows that endomixis occurred at generation 4,103. Thus the first conjugation in 6½ years of culture occurred just after an endomixis. In another place (p. 492), WOODRUFF and ERDMANN report that they found endomixis occurring in some animals of a mass culture while others were conjugating. HERTWIG (1889) observed the same thing; and MÜLLER (1932) found similar phenomena in *P. multimicronucleatum*.

Further indirect evidence of the time relations between the occurrence of conjugation and endomixis is to be found in accounts of the periodicity of conjugation. The two or three week periodicity of conjugation reported by JENNINGS for his stock k is the same as the periodicity of endomixis in the present stock R. If the same periodicity of endomixis characterized JENNINGS' stock, its periodicity of conjugation is readily explained. Similarly, the diverse periodicities of conjugation in the different stocks used by HOPKINS (1921) agree with the differences in interendomictic intervals that characterize different stocks, as will be set forth in a later paper. Likewise, the longer intervals found by HOPKINS between conjugations in *P. caudatum* agrees with the greater interendomictic intervals in that species.

There are still other indications of the time relation between endomixis and conjugation: (1) It is often said that the tendency to conjugate is heightened during periods of depression, which are now well known to be periods of endomixis. (2) The common method of inducing conjugation (JENNINGS 1910) is one which would be expected to induce endomixis just before conjugation. The method consists of bringing about two periods of decline in food supply with an intervening period of abundant feeding. Conjugation is expected to occur during the second decline; the function

of the first decline is probably to induce endomixis. On this view, if endomixis occurred recently, the *first* decline in food supply should yield conjugants; and this has in fact been reported by investigators using this method (SONNEBORN and LYNCH 1934, p. 10; RAFFEL 1930, p. 300).

There are in the literature not only evidences that conjugation occurs at definite times with relation to prior nuclear reorganization, but also evidence that there are times when conjugation cannot be induced. Long observation of any one stock shows that a standard set of inducing conditions will yield conjugants at certain times but not at other times (JENNINGS 1910, HOPKINS 1921, and others). Such observations have led these investigators to suggest that an unknown internal factor plays a role in the determination of conjugation, and this factor may well be the one described in this paper.

What event, process, or substance controls the tendency to conjugate in this stock of *P. aurelia?* Since either conjugation or endomixis serves to put the organisms in a condition in which conjugation can be induced, it may be concluded that the differences between these two processes are not of importance in this connection. Hence, reduction of chromosome number, the introduction of new chromatin from a different animal, and the formation of a syncaryon may be excluded at once. On the other hand, the internal factor that makes conjugation possible must be common to both endomixis and conjugation. There appear to be three such common things from which to choose: (1) disintegration of the old macronucleus; (2) reintegration of a new macronucleus; (3) resorption of the fragments of the old macronucleus and the supernumerary micronuclei. Of these three, two can be eliminated in the light of additional known facts.

The lost tendency to conjugate is restored before animals have reached the stage of endomixis in which resorption and reintegration of the nuclei occur. Many pairs have been observed in which one or both members are in the midst of endomixis. The endomictic "conjugants" are frequently in the stage of endomixis in which anlagen of the new macronucleus have just begun to enlarge and still take practically no chromatic stain; they may even be at the climax of endomixis, fully fragmented but lacking anlagen of the new macronucleus. In such cases, the normal mates show either a whole macronucleus with no fragments, or a macronucleus at the very earliest stage of ribbon formation. These unusual pairs have been found also by others. Some of the earlier mentioned cases of supposed reconjugation of recent conjugants may really be of this type; MÜLLER (1932) definitely states that some of his pairs of *P. multimicronucleatum* contain one member in the midst of endomixis. In my own work, such pairs have occurred under a definite set of conditions. As described above there was in some experiments a period of scanty conjugation, beginning

in some series as early as the eleventh day and lasting in some up to the twentieth day after the last endomixis or conjugation. During this period of scanty conjugation, many animals in a mass culture would go through endomixis at the time when a few others conjugated. In these cultures some of the pairs included animals in the midst of endomixis. What nuclear processes occur in such animals is at present unknown but is under investigation in this laboratory. But the fact that animals in early stages of endomixis will unite as if to conjugate shows that the important thing done by endomixis in preparing animals for conjugation is accomplished early in the process, by the time it reaches the climax. Restoration of the tendency to conjugate must therefore be associated with the prior breakdown of the old macronucleus.

On this view, conjugation is possible so long as an old integrated macronucleus is absent; that is, from the moment it disintegrated, at the climax of endomixis and early in conjugation, until the new macronucleus then formed has lived to be a week or so old. There are two ways of interpreting this relation between the macronucleus and the tendency to conjugate. Either, as the macronucleus ages, it inhibits conjugation; or, when the old macronucleus disintegrates, it discharges into the cytoplasm an enzyme or catalyst which makes conjugation possible. Between these alternatives, a decision can be made on the basis of the relation between rate of reproduction and duration of the tendency to conjugate. There should be a precise inverse proportionality, if the second alternative is correct. Loss of activity by an enzyme or catalyst is presumably due to its dilution below a threshold concentration, and such a point would be reached after a definite number of fissions, irrespective of the time involved. Furthermore, if the tendency to conjugate is dependent upon the disintegration of the old macronucleus, this can be demonstrated by finding the earliest moment after conjugation at which reconjugation is possible in *P. aurelia* and *P. caudatum*. Disintegration of the macronucleus occurs *during* conjugation in the former, and some time *after* it in the latter. Hence, exconjugants should be able to reconjugate at once in *P. aurelia*, but not in *P. caudatum*.

SUMMARY

In stock R of *P. aurelia*, conjugation may be induced by collecting the surplus animals from isolation cultures into fresh culture medium in a small dish and keeping them at 31°C. But not all such cultures conjugate. The determining factor here is the recency of nuclear reorganization. When the culture consists of descendents of animals that have recently undergone endomixis or conjugation, it quickly yields conjugants; but when the culture consists of descendents of animals that have *not* recently

undergone endomixis or conjugation, it will not yield conjugants until after another endomixis has occurred. Response to the conditions favorable for conjugation is of three types, each type characterizing a period of the time following nuclear reorganization. (1) During the first period, varying in extent from the first five to the first twelve days, cultures respond with abundant conjugation; (2) during the second period, extending in some series from the eleventh up to the twentieth day after nuclear reorganization, cultures yield very small proportions of conjugants, or none at all; (3) subsequent to this, in the third period, all cultures completely fail to conjugate but endomixis occurs abundantly instead. Cultures set up in the third period will conjugate readily *after* endomixis has occurred in them.

Not only can two successive conjugations occur with a short interval between them, but long series of successive conjugations are inducible with repeated intervals of only one or a few days. Under certain conditions, individuals in endomixis (climax stage and later) unite as if in conjugation. Hence the process in endomixis that restores the tendency to conjugate must occur early. It must also be the same as a process occurring during conjugation. Disintegration of the old macronucleus is apparently the only process that satisfies these two conditions. A further means of testing the validity of this conclusion is proposed.

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FACTORS DETERMINING CONJUGATION IN PARAMECIUM AURELIA

II. GENETIC DIVERSITIES BETWEEN STOCKS OR RACES

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INTRODUCTION

IN TYPICAL biotypes of the Johns Hopkins stock R of *P. aurelia*, SONNEBORN (1936) has shown that, when surplus animals from isolation lines are collected in a small mass culture and placed at 31°C, conjugation invariably occurs if the animals have recently been through a nuclear reorganization (that is, endomixis or conjugation), but never at other times. In the present paper we contrast the behavior of this stock with that of the Yale stock, cultivated since 1907 by WOODRUFF, which responds very differently to these same conditions.

In *P. aurelia*, racial diversities in the tendency to conjugate have been reported by JENNINGS, WOODRUFF, HOPKINS, and BALL, but their work has been criticized by ENRIQUES, ZWEIBAUM, and the CHATTONS. JENNINGS (1910) found a race k, which was observed to go through 20 epidemics of conjugation in about two years; but during this period race C2 conjugated only a few times, and races c and i only twice. Long continued, systematic and varied attempts to induce conjugation in races c and i invariably failed, but similar attempts in race k were always successful if two or three weeks had elapsed since the last conjugation. WOODRUFF (1912) reported similar results; in some stocks conjugation occurred readily, but in his stock cultured since 1907 conjugation could be obtained only twice, once in 1913 (WOODRUFF 1914) and once in 1920 (WOODRUFF 1921). The most extensive study along these lines was made by HOPKINS (1921). He found marked differences among 13 races, in three respects. (1) Differences in the tendency to conjugate: under similar conditions, some races conjugated frequently, other races only rarely, some not at all. (2) Differences in the conditions required for conjugation: some races conjugated in large stock cultures without special experimental treatment; other races would not conjugate under these conditions, but did so when transferred to small watch glasses with a rapidly diminishing food supply; still other races would not conjugate even under these conditions, but could be made to conjugate when this treatment was preceded by a longer or shorter period of "dormancy." (3) Differences in the periodicity

of conjugation: some races showed no periodicity, others conjugated fairly regularly at intervals of about six weeks, still others at intervals of about three weeks. BALL (1925) added further slight evidence of the same sort, though his main work was on *P. caudatum*.

On the other hand, ZWEIBAUM (1912) and the CHATTONS (1931) have denied the existence of racial differences in the tendency to conjugate. These investigators were able to induce conjugation at will in *P. caudatum* under certain conditions, but not under other conditions; from this they concluded that the diverse results obtained by other investigators on different stocks were actually due to uncontrolled diversities in the environmental conditions. There are, however, several reasons why their conclusion cannot be accepted. In the first place, the production of diversity in the tendency to conjugate by means of diverse environments in one case is no reason to conclude that the same diversity cannot be produced by diversity of constitution in another case. Secondly, even if it could be shown (which, as yet, has not been done) that all or many stocks of a species could be induced to conjugate under one set of conditions, genetic differences might still be demonstrated if, under another set of conditions, some stocks conjugate and others do not. Thirdly, so far as can be learned from their published accounts, ZWEIBAUM (1912) and the CHATTONS (1931) each worked on but a single stock of *P. caudatum*, so they have had no opportunity to encounter racial diversities. Fourthly, the results of the CHATTONS are at variance in a fundamental respect with the results of ZWEIBAUM. ZWEIBAUM found that an essential prerequisite to the induction of conjugation in his stock was a period of starvation ("disette") of 5 or 6 weeks, during which a nutritive equilibrium exists. The CHATTONS, however, found that this was unnecessary in their stock. Thus, the two main proponents of the view that genetic factors play no role in the induction of conjugation have themselves shown that their two stocks differ in the conditions required for conjugation. Finally, HOPKINS (1921) and BALL (1925), using ZWEIBAUM's methods on *P. caudatum* (as well as on *P. aurelia*), were unable to support his conclusion. They found that while his methods were successful with some stocks, they failed with others, and that they were unnecessary in still other stocks, as the CHATTONS also found. Their work, therefore, agrees with the earlier work of JENNINGS (1910), WOODRUFF (1912), and CALKINS and GREGORY (1913) on the same species, as well as the papers cited in the preceding paragraph on *P. aurelia*. In addition to these investigations on *P. aurelia* and *P. caudatum*, WENRICH and WANG (1928) presented evidence for racial differences in tendency to conjugate in *P. calkinsi*.

Thus ZWEIBAUM's contention has been experimentally disproved. That of the CHATTONS is in little better case, but an investigation directed pre-

cisely upon the point which they make, and that shall adequately test it, will be worth while. Such an investigation is here presented. The CHATTONS maintain that differences in bacterial flora are the basis of apparent racial differences in tendency to conjugate. It is required, therefore, to compare different stocks under identical bacterial conditions.

EXPERIMENTAL RESULTS

For this purpose, we selected two stocks reported to be very diverse in their tendencies to conjugate. The first was the Johns Hopkins stock R in which conjugation could invariably be produced under the conditions described by SONNEBORN (1936). The second was the Yale stock in which conjugation has occurred only twice in 29 years (WOODRUFF 1921). (We are grateful to Professor Woodruff for his kindness in sending us a strain of this stock.) The carefully controlled method of cultivation, using but one species of bacterium and one species of alga, and the methods of inducing and detecting conjugation and endomixis were all fully described by SONNEBORN (1936) and need not be repeated here.

The two stocks were compared, under identical environmental conditions, in the following way. A large group of daily isolation lines of each stock was cultivated on pyrex depression slides from February 15 until March 30, 1935. Each day the surplus animals from the isolation cultures were collected into small mass cultures, which were kept at 31°C and observed for conjugants at not more than 12 hour intervals. All mass cultures were observed for at least five days (when multiplication had practically stopped); and those cultures in which 75 percent or more of the animals went into endomixis were fed again and observed for at least four more days until after multiplication had again practically stopped. Altogether, there were under observation 179 mass cultures of the Yale stock and 187 mass cultures of the Johns Hopkins stock R. *Not a single pair of conjugants could be found in any of the cultures of the Yale stock; but conjugation occurred in every one of the cultures of the Johns Hopkins stock R.*

Although both stocks were cultivated under identical conditions and although pure cultures of but one species of bacterium (*Flavobacterium brunneum*) were inoculated into the culture fluid daily, it was still possible that different contaminating species of bacteria had gotten into the cultures of the two stocks. To equalize such contamination, if it had occured, lines of the Johns Hopkins stock R were transferred to fluid in which the lines of the Yale stock had lived for 24 hours, and vice versa. During the days following this exchange of fluids, 57 mass cultures were prepared as before. The results with these cultures were exactly the same as before: *all cultures of the Johns Hopkins stock R conjugated, but none of the cultures of the Yale stock did.* Their difference in the tendency to conju-

gate cannot, therefore, be due to differences in bacterial flora. The clear-cut, constant, and many times repeated difference found between these two stocks demonstrates definitively that they differ genetically in their tendency to conjugate. Under the same environmental conditions, they invariably respond differently. The criticism of the CHATTONS, like that of ZWEIBAUM, is thus unfounded, and racial differences in tendency to conjugate do in fact exist within a single species of Paramecium.

SUMMARY

Under carefully controlled environmental conditions, the Johns Hopkins stock R of *P. aurelia* can invariably be induced to conjugate; under identical conditions, the Yale stock of the same species cannot. The contention of the CHATTONS (1931) that such differences are not racial, but due to differences in bacterial flora, is shown to be invalid.

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STUDIES ON THE INHERITANCE OF PERSISTENCY[1]

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INTRODUCTION

THE ability to lay late in the fall at the close of the first laying year is very desirable in breeding for egg production. GOODALE and SANBORN (1922) pointed out that high persistency is one of the essential characters in heavy laying Rhode Islands Reds. HAYS and SANBORN (1926) showed an intimate correlation between persistency and egg production. HAYS and SANBORN (1927a) found the net correlation between persistency and annual production to be higher than between any other fecundity character studied and annual production. HAYS and SANBORN (1933) also report that breeding from females with persistency greater than 365 days gave daughters having superior fecundity. JULL (1932) also showed the importance of high persistency in relation to egg production.

Studies on the mode of inheritance of persistency are very limited. HURST (1925) stated that high persistency behaves as a simple recessive to low persistency. HAYS (1927b) presented data on the inheritance of high persistency in Rhode Island Reds. In this early study on production-bred birds, the dividing point between high and low persistency was arbitrarily placed between 314 and 315 days from first pullet egg. Persistency was also limited to a 365 day period following the first pullet egg. The studies reported here include the production-bred birds hatched from 1917 to 1930, the exhibition-bred birds hatched in 1930 and F_1 and F_2 hybrids and backcrosses hatched in 1930, 1931, 1932, and 1933. In these later studies the persistency period may continue beyond the 365-day laying year until the individual shows a 30-day cessation of production which is assumed to indicate the annual molt. Persistency within the first laying year may likewise be terminated by a pause of thirty days or more.

CHARACTER OF THE DIFFERENT POPULATIONS

A total of 910 production-bred pullets hatched from 1917 to 1930 may be considered a representative sample of the production stock used for hybridization. Figure 1 shows the frequency distribution of this population with respect to length of biological year. The data fail to show a normal distribution unless five individuals with persistency between 185 and 214 days are omitted. Omitting these five, the distribution by the χ^2 test gives a probability of .2498 which means that in one case out of four a random sample would give deviations as great as or greater than those observed.

[1] Published as Contribution No. 251 of the Massachusetts Agricultural Experiment Station.

FIG. 1

BIOLOGICAL YEAR - DAYS

910 PRODUCTION - BRED BIRDS

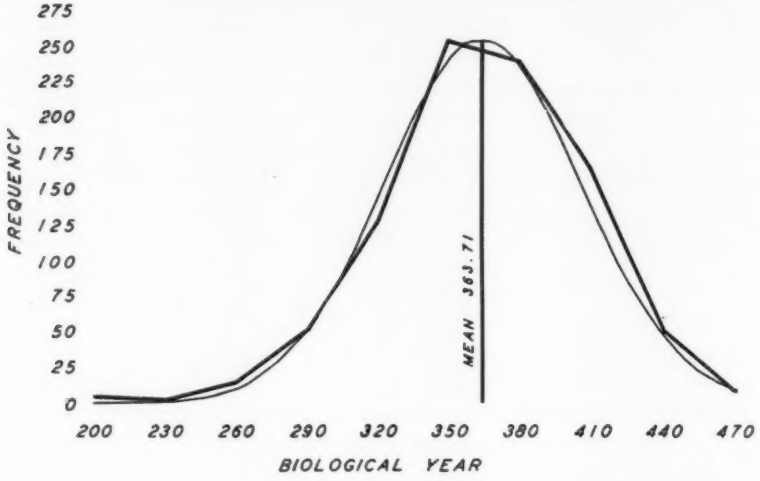
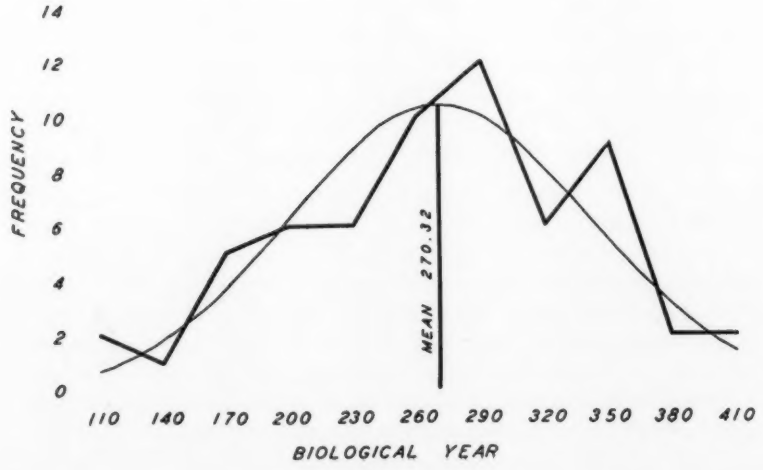


FIG. 2

BIOLOGICAL YEAR - DAYS

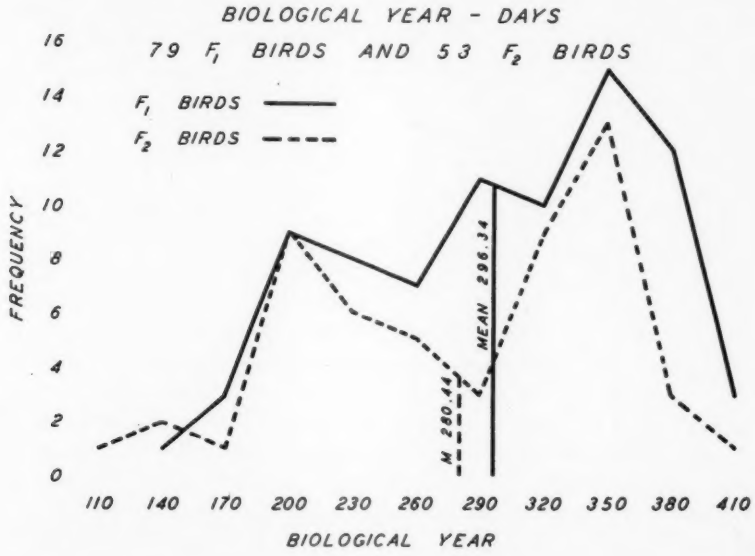
61 EXHIBITION BIRDS - 1930



The generation of exhibition birds hatched in 1930 consisted of 61 individuals with a mean biological year of 270.32 days. Figure 2 gives the frequency distribution of this population for biological year. This distribution is very close to normal and the χ^2 test for normalcy gives a value of P of .6655.

First and second generation crosses are presented in figure 3. The frequency distribution for the F_1 generation shows a wide scatter as might be anticipated when a cross is made between two lines neither of which is homozygous for the character in question. There is an indication, however, of the dominance of high persistency.

FIG. 3



The F_2 generation clearly shows a bimodal distribution for length of biological year with modes at about 200 days and 350 days. The graph demonstrates that two populations with rather characteristic length of biological years are concerned. An inspection of this graph suggests that the dividing point between the two populations lies in the neighborhood of 270 days.

Figure 4 gives the frequency distribution of the 23 F_2 birds that had a biological year below 270 days. This population has a mean of 203.63 days and the χ^2 test for normalcy of distribution gives the value of P as .3885 which is satisfactory evidence that the distribution is normal.

FIG. 4

BIOLOGICAL YEAR - DAYS

23 F_2 BIRDS - UNDER 270 DAYS

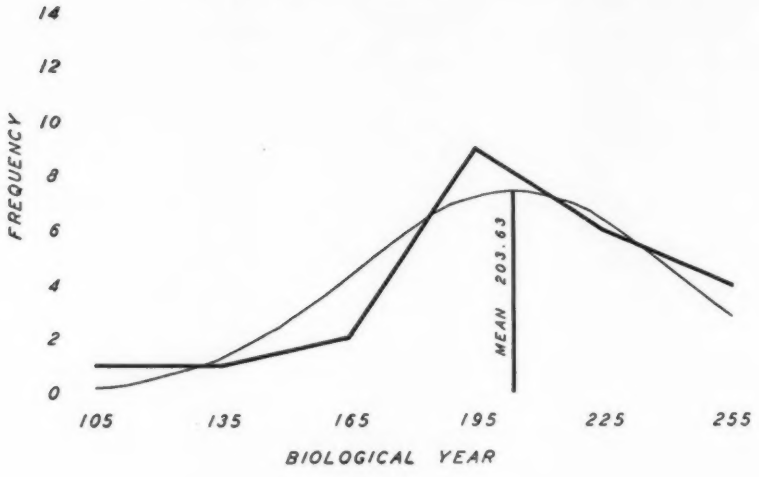


FIG. 5

BIOLOGICAL YEAR - DAYS

30 F_2 BIRDS - OVER 270 DAYS

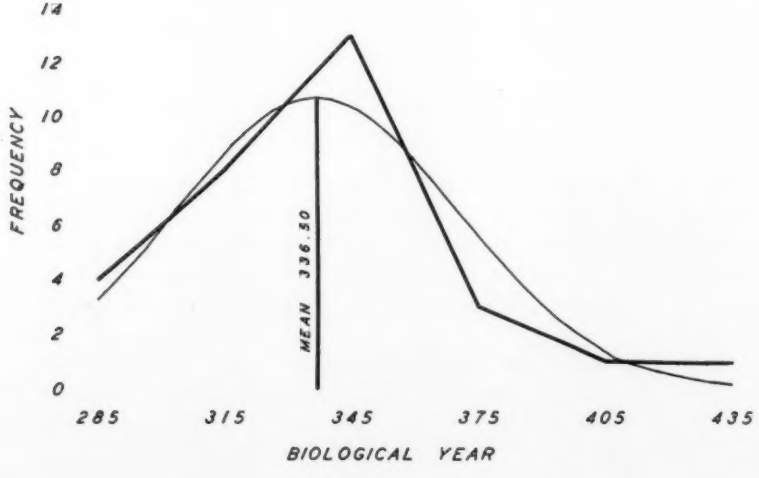


Figure 5 includes the 30 F_2 birds with a biological year of 270 days or more. The mean persistency of this population is 336.50 days. By the χ^2 test the value of P is .2343 showing that this group of persistent layers represents a normal distribution. The fact should be stressed in this connection that the arbitrary separation of the F_2 population into low and high persistency classes at 270 days will operate to include some individuals around this dividing point in the wrong phenotype.

RESULTS FROM CROSSES

For analytical and practical purposes the dividing point between high and low persistency phenotypes appears to occur at close to 270 days. The former figure of 315 days used by HAYS (1927) is not substantiated in crosses where an essentially low persistency line of exhibition birds is crossed with a highly persistent line of production birds.

Some specific examples of the ratios may next be considered. In 1930 an exhibition male No. J711 was mated to four production females all of which were phenotypically highly persistent. From these matings 45 F_1 daughters have complete persistency records. The data show 24 highly persistent to 21 low in persistency with an expectation of equal numbers if one dominant gene is concerned. The male evidently lacked gene *P* and the females were heterozygous. This male was shown to lack the gene for high persistency by mating to his F_1 daughters. Unfortunately only a small number of daughters from three matings had complete persistency records. These showed 2 high to 5 low where the expectation was 3.5 to 3.5. The maternal backcross obtained by mating the original production females to an F_1 son gave 10 high to 2 low where the expectation was 9 to 3. A cross of production males to exhibition females that were phenotypically high gave 23 F_1 daughters in the proportion of 19 high to 4 low where the expectation was 17.25 to 5.75. A production male K56 mated to F_1 females that were high in persistency gave 20 high to 4 low where the expectation was 18 to 6.

A production male mated to persistent F_2 females gave 15 high to 4 low daughters and the expectation was 14.25 to 4.75. An exhibition male mated to persistent F_2 females gave 8 high to 4 low where the expected was 9 to 3.

One exhibition male, K718, when mated to low persistency hens gave proportions of highly persistent daughters indicating that he carried one gene for high persistency. Reciprocal crosses between the two lines gave the same results confirming earlier findings at this Station that high persistency depends in inheritance upon a single dominant autosomal gene *P*.

SUMMARY

Results from crossing high and low persistency lines of Rhode Island Reds show that the dividing point between high and low persistency phenotypes lies close to 270 days. That high persistency depends in inheritance upon a single dominant autosomal gene is confirmed. In breeding operations it is recommended that the standard for selecting breeding stock for high persistency be placed well above a 270 day minimum because of overlapping in phenotypes.

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THE INHERITANCE OF TAILLESSNESS (ANURY) IN THE HOUSE MOUSE

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INTRODUCTION

THE short-tailed (Brachyury) mutant form of the house mouse, discovered by DOBROVOLSKAIA-ZAWADSKAIA (1927a), behaves as a dominant to the normal or wild type. The homozygotes show characteristic abnormalities (CHESLEY 1932, 1935) and die *in utero* about eleven days after fertilization.

From the original short-tailed (Brachy) stock following outcrosses to wild mice, three substrains have arisen, each characterized by complete absence of the bony tail (anury). These have been described by DOBROVOLSKAIA-ZAWADSKAIA and KOBOZIEFF (1927b). These authors (1932) and KOBOZIEFF (1935) have reported that tailless mice of each of these strains produce only tailless mice when bred to members of the same strain. Crosses between members of different strains are said to produce both tailless and normal progeny, although complete data on such crosses have not been published. Because of this peculiar behavior, ZAWADSKAIA and KOBOZIEFF suggested that the tailless strains may contain balanced lethals. This suggestion rested on analogy, since from the evidence available it was not possible to identify the lethals involved nor to specify what conditions held the lethals in association.

Our interest in the developmental action of lethal genes led us to undertake a further analysis of the tailless strains in order to isolate the genes involved and to study their effects on the embryos. The results of this analysis show that mice of one of the tailless strains (Agouti or A line of

[1] These experiments were being carried on in this laboratory by Dr. Chesley, and most of the observations (except those on line 29) were made by him. Before Dr. Chesley's death in February 1936, we had had frequent consultations on the results, and I was sufficiently familiar with the data and with Dr. Chesley's working hypotheses to be able to continue the experiments as originally planned. Although the major part of the data and of the interpretations had been obtained by Dr. Chesley, he had not intended to publish them until after further evidence had been obtained concerning (1) the morphological characters of the new type of dead embryos found (t^0t^0 p. 530), (2) the difference in the segregation ratios from males and females containing the t^0 gene which he thought required an examination of the spermatogenesis of T/t and $+/t^0$ males, (3) the possibility of crossing over between T and t^0 since he was not convinced that these genes were alleles. Since some time may elapse before the first two problems can be adequately studied, I have thought it best to record the results obtained and to assume responsibility for the form of publication and the conclusions drawn. I wish, too, to record my respect and admiration for Dr. Chesley as student, colleague and friend, and my regret that a scientific career that had begun so auspiciously should have ended so early. L. C. D.

ZAWADSKAIA) are heterozygous for two lethals, one the previously known Brachyury (T), the other a recessive (t^o) which in combination with T produces taillessness. T and t^o are probably alleles or so located as to show no crossing over. In this report we shall summarize the genetic analysis of this tailless strain. Preliminary evidence is reported showing that t^o homozygotes die very early in development, shortly after implantation. It is hoped that a more complete description of the morphology and development of the new lethal type can be made later.

MATERIALS

Dr. ZAWADSKAIA kindly brought stock of all three strains to our laboratory in 1933 and placed them at our disposal. We are greatly indebted to her for her cordial cooperation in our work.[2] Because of difficulties encountered in maintaining the stocks, it was necessary to outcross two of them (lines 29 and 19, cf. KOBOZIEFF 1935) to normals and Brachys of a stock previously supplied by Dr. ZAWADSKAIA and to recover the strains by backcrossing the outcross animals to the ZAWADSKAIA tailless strains followed by inbreeding. Although we recovered true-breeding tailless stocks of each of these, it is not certain that the genetic constitution of each is identical with that of the original stocks. The third tailless stock (A line) is descended from Dr. ZAWADSKAIA's stock without outcrossing.

BREEDING EXPERIMENTS

Our first experiments confirmed the reports of ZAWADSKAIA and KOBOZIEFF that each of the tailless lines bred true and was characterized by small litters (table 1). The progeny from lines A and 19 were all completely tailless, no vertebrae having been noted (by palpation) posterior to the

TABLE 1

Results of matings of tailless lines inter se and of crosses between different tailless lines.

PARENTS	REFERENCE	OFFSPRING			LITTER SIZE
		TAILLESS	BRACHY	NORMAL TAIL	
A × A	This paper	239			3.6
A × A	KOBOZIEFF (1935)	*63*			*2.7*
29 × 29	This paper	167			3.8
29 × 29	KOBOZIEFF (1935)	*194*			*4.1*
19 × 19	This paper	63			3.9
19 × 19	KOBOZIEFF (1935)	*34*			*3.4*
A × 19	This paper	9		9	3.6
A × 29	This paper	23		15	5.7
A × 29	KOBOZIEFF (1935)	*14*		*15*	5.8

[2] For later shipments we are also grateful to Dr. Walter Landauer and Dr. Boris Ephrussi who brought mice to us from Dr. ZAWADSKAIA's laboratory.

sacrum. In line 29, we have found occasional animals with a small bony stump, never containing more than 2 or 3 tail vertebrae. In all lines there is usually a short "tail filament" consisting of skin and connective tissue, probably the remains of the tail which is formed in early embryonic development, but which is destroyed and resorbed, as described for the short-tailed mice (CHESLEY 1934). Many of the animals in each line are sterile, small and mature slowly.

Inheritance of Taillessness in Line A

The first problem was to determine the genetic constitution of the tailless animals in each strain. Reciprocal crosses were made between tailless animals and normals from an inbred stock (Bagg albinos) and between tailless and short-tailed (Brachy) animals of the ZAWADSKAIA parent stock. The progeny of these crosses were then tested and bred in various ways. Only one strain (line A) has been analyzed in detail, and conclusions, for the present, must be limited to the tailless condition as found in this strain. There are indications that the main features of the inheritance of taillessness are similar in the other strains.

The data for line A crosses are shown in table 2. Observations were all made as near birth as possible, usually within 12 hours of parturition. The distinction between the three classes, normal tail (nt), short tail (Brachy or Br), and tailless (zero tail or Ot) can be made at birth with little uncertainty. In the tailless animals there is no caudal skeleton at all, and the tail filament, which is nearly always present, hangs limply from the end of the sacrum; in short-tailed animals there is a stiff cartilaginous tail of from $\frac{1}{8}$ to $\frac{7}{8}$ of the length of the normal, ending bluntly, usually with a thin thread-like filament attached, the remnant of the resorbed posterior end of the embryonic tail; in normals the tail tapers to a point and never shows a filament.

Crosses of line A tailless and normal. The cross of tailless (line A) and normal (inbred Bagg albino) gives regularly two types of offspring, normal and Brachy. When the tailless parent is the mother, this segregation occurs in a 1:1 ratio (table 2, Exp. 1). When the tailless parent is the father, there is a considerable excess of normal offspring (Exp. 2), a difference which appears whenever taillessness is introduced through the male and which requires detailed discussion later.

The F_1 Brachy offspring behave like ordinary Brachy animals of the ZAWADSKAIA Brachy stock. When bred together or crossed with other Brachys, they produce only Brachys and normals (Exp. 10), and the homozygous Brachy type of lethal embryo is found in the uterus at the 10th day and dies shortly thereafter. When bred to normals, they produce Brachys and normals in a 1:1 ratio (Exp. 11). They do not transmit the

tailless condition as found in the tailless parent line (11 females and 6 males thoroughly tested by Brachys from stock) and are evidently of the constitution $T/+$, like the usual Brachy animals. This shows that the A tailless parent is heterozygous for Brachyury.

TABLE 2

Results of matings of tailless mice of line A and of tests of their descendants.

EXP. NO.	♀	PARENTS ♂	GEN.	NO. OF MATINGS	TOTAL YOUNG	LITTER SIZE	OFFSPRING		
							NORMAL	TAILLESS	BRACHY
1	Ot	Normal*	F_1	20	114	5.7	63		51
K‡	Ot	Normal	F_1	5	25	5.0	12		13
2	Normal*	Ot	F_1	30	179	6.0	140		39
K‡	Normal	Ot	F_1	9	46	5.1	46		
3	Ot	Brachy	F_1	5	24	4.8	7	11	6
4	Brachy	Ot	F_1	37	304	8.2	128	127	49
5	F_1 nt (from 1)	Brachy	TC	47	335	7.1	164	87	84
6a	Brachy	F_1 nt (from 1 and 2)	TC	65	484	7.4	242	171	71
6b†	Brachy	F_1 nt (repetition)	TC	124	935	7.5	491	297	147
7	F_1 nt (fr 1)	Normal*	BC_1	22	144	6.6	144		
8	F_1 nt (fr 1)	F_1 nt (fr 1)	F_2	12	80	6.7	80		
9	F_1 nt (fr 1)	Ot (stock)	BC	19	89	4.7	53	22	14
10	F_1 Brachy (fr 1)	Brachy (stock)	TC	41	229	5.6	90		139
11	F_1 Brachy (fr 1)	Normal*	BC	10	53	5.3	33		20
12	F_1 Brachy	Ot (stock)	BC	8	52	6.5	21	27	4
13	$BC_1 +/t^0$ (fr 7)	Brachy	TC	34	200	5.8	90	55	55
14	Brachy	$BC_1 +/t^0$ (fr 7)	TC	20	169	8.5	85	47	38
15	$BC_1 +/+$ (fr 7)	Brachy	TC	42	295	7.2	149		146
16	Brachy	$BC_1 +/+$ (fr 7)	TC	10	91	9.1	51		40
17	$BC_1 +/t^0$ (fr 7)	Normal*	BC_2	7	45	6.4	45		
18	$F_2 +/t^0$ (fr 8)	Brachy	TC	20	127	6.4	57	37	33
19	Brachy	$F_2 +/t^0$ (fr 8)	TC	12	85	7.1	35	26	24
20	Brachy / $F_2 +/+$ (fr 8)	$F_2 +/+$ (fr 8) / Brachy	TC	22	158	7.2	79		79
21	$BC_2 +/t^0$ (fr 17)	Brachy	TC	5	34		16	9	9
22	Brachy	$BC_2 +/t^0$ (fr 17)	TC	12	100	8.3	53	32	15
23	Brachy	$BC_3 +/t^0$	TC	25	222	8.9	118	77	27
24	$BC_3 +/t^0$	Brachy	TC	9	53	5.9	27	14	12

‡ Data from KOBOZIEFF (1935).

* All normals were from our inbred stock of Bagg albinos.

† Offspring from two males omitted; cf. table 5.

The F_1 normal-tailed animals all transmit taillessness (31 males and 29 females thoroughly tested). This is best revealed by crossing F_1 normal females to Brachy males from stock. This produces about 2/4 normals, ¼ Brachys, and ¼ tailless (Exp. 5). Normals not derived from tailless produce, when crossed with Brachy, only Brachy and normal progeny. This shows that the tailless parent has transmitted a recessive gene for taillessness, which we may call t^0, to all normal-tailed F_1 progeny, thus

proving the tailless parent to have been heterozygous both for T and for t^0. We may therefore assume that A tailless animals are T/t^0, F_1 Brachys $T/+$, and F_1 normals $t^0/+$. Taillessness is assumed to arise from interaction between T and t^0.

The constitution of the F_1 normals was also tested in several other ways. F_1 females were backcrossed to the tailless parent. This produced normal-tailed, Brachy and tailless progeny (Exp. 9).

F_1 normal females backcrossed to the normal parent stock produced only normal progeny (Exp. 7). These BC progeny, when tested by crossing, to Brachy, fell into two groups. One group (26 thoroughly tested) produced only Brachys and normals and were thus $+/+$ (Exps. 15, 16); the other group (39 thoroughly tested) produced normals, Brachys and tailless and were thus $+/t^0$ (Exps. 13, 14). The latter were again backcrossed to normal parent stock (Exp. 17), and the normal progeny so produced were again shown to consist of $+/+$ (7 tested) and $+/t^0$ (9 tested, Exps. 21, 22). The latter were again backcrossed to normal (Bagg) stock and the BC_3 progeny when tested fell into two groups: $+/t^0$ (Exps. 23 and 24) and $+/+$. By thus successively backcrossing normal animals, which were known to transmit taillessness, to the same inbred normal stock, any other genes affecting tail form tended to become homozygous, and the clear segregation of only two genetic types, $+/+$ and $+/t^0$, shows that a single gene, t^0, is involved.

F_1 normals bred together produced only normals (Exp. 8), but these F_2 normals, when tested by Brachys, fell into two groups, one of which (17 tested) produced tailless progeny in addition to normal and Brachy (Exps. 18, 19), while the other (9 tested) produced no tailless progeny (Exp. 20).

Thus the gene responsible for taillessness was shown to be transmitted through normal animals for four generations, to produce no effect on the tail when in combination with a normal allele, but to produce typical taillessness when again combined with T.

Crosses of line A tailless by Brachy. A direct test of the assumed constitution of the A tailless females was made by crossing them with Brachys from Brachy stock. If our asumptions are correct, this cross should have the following results:

A tailless × Brachy			
T/t^0	$T/+$		
T/T	T/t^0	$t^0/+$	$T/+$
dies	tailless	normal	Brachy

The expected classes were obtained, although, because of infertility of tailless females, the numbers are as yet too small to establish the ratio with certainty (Exp. 3). The reciprocal cross of tailless male made by

Brachy female yielded tailless, normal and Brachy progeny (Exp. 4, 12), as discussed below.

This may be taken as confirming our assumption that tailless animals are T/t^0. What then explains the fact that such hybrids, when bred together, produce only one type of offspring? Two assumptions need to be added: (1) that the homozygote t^0t^0 is inviable; (2) that T and t^0 are either alleles or else crossing over between them is prevented by close linkage or by some other means. Since all our data are consonant with the allele interpretation we shall use this until disproved. Matings of A tailless by A tailless would then be:

$T/t^0 \times T/t^0$

T/T	T/t^0	t^0/T	t^0/t^0
Brachy homozygote dies 10–11 days	tailless		tailless homozygote dies early

Litter size. If this is correct, litters from $T/t^0 \times T/t^0$ should be 50 percent smaller than litters from $T/t^0 \times +/+$, and should contain two groups of dead embryos: one, the Brachy homozygous type already studied; another, a new type due to the combination t^0t^0. Litter size may be influenced by factors other than the action of lethal genes (residual heredity of stocks, age of mother, ordinal number of litter, etc.), so that comparisons of litter size may not provide crucial evidence of the number of lethals segregating. In the present case, 66 liters from A tailless by A tailless contained 239 young, an average of 3.6 per litter. The average size of 50 litters from tailless by normal (Bagg albino) was 5.9. The former is 60 percent of the latter; it is smaller than the 75 per cent expected if only one lethal were segregating in tailless by tailless matings.

EVIDENCE FROM EXAMINATION OF EMBRYOS

Direct evidence of two lethals was obtained from dissections of the uteri of tailless females pregnant by tailless males (table 3). Examinations were made usually on the 11th day after timed copulations. Three types of embryo were found: (1) living embryos representing the viable tailless class; (2) abnormal embryos identical with the previously recognized and studied homozygous Brachy type (CHESLEY 1935); these were either still alive or had recently died; (3) embryos which had died shortly after implantation, consisting only of a small mass of resorbing material. These masses were of remarkably uniform size, indicating that the group as a whole had died at about the same time. The swellings of the uterus containing these masses were smaller than those containing normal or homozygous Brachy embryos. Of

the resorbing masses dissected at the 11th day, each contained a well developed placenta with a completely resorbed embryo. Some uteri (tailless ×tailless) were fixed entire on the 6th, 7th, and 8th days after copulation, and the embryos were then dissected out and sectioned. Preliminary examination of these shows that resorption of the abnormal type begins probably on the 6th and is certainly complete or nearly so by the 8th day. Eighth day dissections showed 39 normal, 24 resorbed and four swellings in which it could not be determined whether embryonic material had been present or not.

TABLE 3

Embryos found on dissection of uteri of pregnant females (from timed copulations).

MATING	MOTHER	FATHER	MOTHERS DISSECTED	EMBRYOS				VIABLE EMBRYOS PER LITTER
				VIABLE	BRACHY HOMOZYGOTES TT	RESORBED t^0t^0	?	
1	AA tailless	AA tailless	24	73	22	65	6	3.0
2	Normal (Bagg)	AA tailless	2	20	—	—	3	10.0
3	Brachy	AA tailless	14	114	12	3	9	8.1
4	*Normal $+/t^0$	Normal $+/t^0$	19	102	—	58	8	5.4

* F_1, BC and F_2 animals from tailless by normal, which had been tested and found to be $+/t^0$

The resorbed group probably contains the t^0t^0 class, although it may contain also a few embryos which died after implantation from other causes. As controls, normal females pregnant by tailless males were dissected. Out of 23 embryos none of the resorbing type was found. In the case of three capsules no decision concerning the presence of embryonic tissue could be made. Likewise Brachy females pregnant by tailless males were dissected. About 10 per cent of the embryos were of the homozygous type, again proving that tailless males transmit brachyury. Three out of 138 embryos appeared to be of the resorbed type (table 3, mating 3), although these were not sectioned and the diagnosis was indicated as doubtful. Certainly there is no approach here to the frequency of the resorbed type found among tailless by tailless matings.

The data from dissections agree well with those from litters observed at birth. Thus in tailless by tailless matings the average litter size was 3.6 (table 1, Exp. 1); at the 11th day the average number of viable embryos was 3.0 per litter. The average number of embryos implanted was 6.9 of which 56.5 percent died after implantation as compared with 50 percent expected to die if two lethals are present.

F_1 normal-tailed females ($+/t^0$) from the cross to normal (Bagg albino) pregnant by their brothers ($+/t^0$), when dissected in the 10th day of pregnancy, showed 35.6 percent of resorbing embryos of similar size to those

found in the uteri of tailless females (table 3, mating 4). This group probably contains the t^0t^0 lethal class expected, but the proportion is higher than the 25 percent expected. A few $+/t^0$ females from BC_1 and F_2 pregnant by $+/t^0$ males showed a similar proportion of the resorbing class.

ALLELISM OF T AND t^0

The results described above are consistent with the assumptions that the tailless line studied is heterozygous for the previously known dominant T, and for a recessive lethal t^0 which, when present with T, results in taillessness. These appear not to cross over. Crossing over would result in gametes without either gene which, when fertilized (in *inter se* tailless matings) by a T gamete, would result in Brachy offspring or, by t^0 gametes, would give normal offspring. Neither of these has been observed among 239 offspring of tailless × tailless in our experiments or among 63 offspring reported for the same line by KOBOZIEFF (1935). Likewise 60 F_1 normals from tailless by normal were shown to be $t^0/+$, indicating no crossing over in 60 gametes of tailless. It is probable then that T and t^0 are alleles and that these two lethals are balanced in the tailless line by their allelism. If this is so, then two alleles, each of which is lethal when homozygous, are not lethal when combined with each other (T/t^0), a situation which is sufficiently unique to be regarded with some suspicion as a final explanation. If it proves to be correct, then it will show that the dominant allele T is not a deficiency, since when combined with a lethal t^0 opposite to it, a viable embryo results. The non-lethal nature of the compound T/t^0 suggests that these alleles influence sufficiently different physiological processes so that a defect in one is made good by the other. The probable existence of another lethal allele in other tailless lines (see below) which in combination with t^0 leads to normal development points in the same direction. Further discussion is deferred until a more rigid test of allelism is obtained.

EXCESS OF TAILLESS OFFSPRING FROM t^0 FATHERS

The hypotheses proposed fit the facts obtained, when t^0 is introduced from the mother. When the father contains t^0, however, the proportions of offspring containing t^0 show significant departures in excess of the expected. Tailless females crossed with normal (Bagg) males produced 63 normals ($+/t^0$) and 51 Brachys ($+/T$); the reciprocal cross gave 140 normals and 39 Brachys. Normal females heterozygous for t^0 (from F_1, BC_1, BC_2 and BC_3), when crossed with Brachy males, gave the results shown in table 4 as compared with those expected on the above assumptions. Comparable matings of $+/t^0$ males by Brachy females gave the results in table 5.

TABLE 4

PARENTS		OFFSPRING NORMAL +/+; +/t^o	TAILLESS T/t^o	BRACHY $T/+$
F_1 ♀ +/t^o×Brachy +/T♂		164	87	84
BC_1 ♀ +/t^o×Brachy +/T ♂		90	55	55
BC_2 ♀ +/t^o×Brachy +/T ♂		16	9	9
BC_3 ♀ +/t^o×Brachy +/T ♂		27	14	12
F_2 ♀ +/t^o×Brachy +/T ♂		57	37	33
Totals	actual	**354**	**202**	**193**
	expected 2:1:1 ratio	*374.5*	*187.2*	*187.2*
Sex ratios (per cent ♂♂)		47.2	58.0	50.9

TABLE 5

PARENTS	OFFSPRING NORMAL	TAILLESS	BRACHY
F_1 ♂ +/t^o×Brachy ♀ (1st test)	242	171	71
F_1 ♂ +/t^o×Brachy ♀ (repetition) (six Type 1 males only)	491	297	147
BC_1 ♂ +/t^o×Brachy ♀	85	47	38
BC_2 ♂ +/t^o×Brachy ♀	53	32	15
F_2 ♂ +/t^o×Brachy ♀	35	26	24
BC_3♂×Brachy ♀	118	77	27
Total	**1024**	**650**	**322**
Expected 3:2:1 ratio	*998*	*665.3*	*332.6*
Sex ratios (per cent ♂♂)	45.8	54.9	48.7
F_1 ♂ +/t^o×Brachy ♀ (two type 2 males only)	159	124	34
Sex ratios (per cent ♂♂)	46.5	56.5	45.6

These results clearly establish that +/t^0 males produce a significantly higher proportion of tailless progeny when tested by Brachy than do +/t^0 females. The +/t^0 males in table 5 are sibs of the +/t^0 females in table 6 and the Brachy animals used were all from the same Brachy stock.

Moreover, different +/t^0 males appeared to give different proportions of tailless progeny. A special test of this point is in progress (repetition of F_1 ♂×Brachy, table 5). Eight F_1 +/t^0 males have been tested by crossing with Brachy females. Six of these have each produced normal, tailless and Brachy progeny in a ratio approximating 3/6 normal; 2/6 tailless; 1/6 Brachy (actually 491:297:147). Two males each gave very few Brachy offspring (actually 159 normal:124 tailless:34 Brachy). The existence of two types of +/t^0 males indicates that another factor or factors are involved and these are being studied further. The difference is not due to the source of the t^0 gene since males receiving it from the mother produce progeny in the same proportions as those receiving it from the father.

Omitting the results from males shown to be of the second type, the ratios as a whole give a good fit to a 3:2:1 ratio.

The departure of this from the 2:1:1 ratios regularly obtained from $+/t^0$ females is probably not due to sex-linkage. The t^0 gene is certainly not sex-linked, since it is transmitted regularly to both sexes by T/t^0 and by $+/t^0$ males and females. No marked variations in the sex ratios of the three types of progeny obtained from crosses of these genotypes by Brachy females have been observed (tables 4 and 5). The slight excess of males among the tailless progeny may be due to differential viability.

The peculiarity in inheritance of t^0 is its more frequent transmission through the sperm than through the egg. Most males of the type $+/t^0$ appear to produce about twice as many t^0 as $+$ sperm, as inferred from the production of twice as many T/t^0 as $T/+$ progeny when crossed with Brachy. Such a condition might arise if t^0 spermatocytes were to undergo one further equational division although we have no direct evidence on this.

A similar excess of offspring with t^0 is found wherever t^0 is introduced from the male. Thus tailless males by Brachy females gave 121 normals ($+/t^0$), 46 Brachy ($+/T$) and 122 tailless (T/t^0); the tailless progeny outnumber the Brachys by over two to one, equality expected. However, normal (Bagg albino) females by tailless males gave 140 normal ($+/t^0$) and only 39 Brachys ($+/T$), a wide departure from a 2:1 ratio. Here again individual tailless males may give different ratios and this must be tested in new experiments. KOBOZIEFF (1935) also crossed normal females from a laboratory stock of albinos with A tailless males and obtained 46 *normals* only. His reciprocal cross gave 12 normals and 13 Brachys; ours gave a similar result, 63 normals and 51 Brachys. KOBOZIEFF attributed the difference to genetic differences in the normal animals used in the reciprocal crosses; but this cannot be true in our case, since we have used only animals from the 40th–50th brother-sister generations of our stock of Bagg albinos. The differences are probably due to peculiarities in the transmission of t^0 by males.

The ratios of non-viable embryos reveal a similar distortion whenever t^0 is introduced through the male. Thus dissections of tailless females pregnant by tailless males gave unexpected ratios as follows:

T/t^0 ♀ × T/t^0 ♂	FOUND PERCENT	EXPECTED (NORMAL SEGREGATION)	EXPECTED (2 t^0:1 T SPERM)
Brachy homozygotes TT (dead)	13.8	25	16.6
Early resorption t^0t^0 (dead)	40.6	25	33.3
Viable T/t^0	45.6	50	50

The data do not fit the ratio expected if both eggs and sperm segregate in 1 T:1 t^0 ratios; the fit is better, although still is not perfect to the assumption of a 1:1 ratio among the eggs but a 2 t^0:1 T ratio among the sperm.

Similarly, in crosses of tailless male (T/t^0) by Brachy female ($T/+$) 25 percent of TT embryos are expected. This class is readily recognized and the classification is accurate. Actually only 10 percent were found in this cross, which correlates fairly well with the deficiency of Brachys ($+/T$) and the excess of tailless (T/t^0) among the progeny born (table 2, Exp. 4). Finally the dissections of matings of $+/t^0 \times +/t^0$ (F_1 normals from tailless×normal) showed 35.6 percent of the early lethal type t^0t^0, which is to be compared with 25 percent expected if segregation is normal in both sexes or with 33.3 percent if sperms segregate in 2 t^0/1 + ratio. Because of wide variations in different individual matings, this question will require further study.

TAILLESSNESS IN OTHER LINES

It has not yet been possible to complete a comparable analysis of the inheritance of taillessness in lines 29 and 19. Preliminary evidence however is sufficient to show that each of these lines is heterozygous for T and for some other recessive condition like t^0 which, in combination with T, produces taillessness. The additional gene in line 29 is probably not the same as that which occurs in line A, since these two lines when crossed produce both tailless and normal progeny (table 1). In our cross these occurred in a ratio of about 2:1. This would result, if line 29 carried an allele such as t^1 of the t^0 gene of line A. The normal progeny from the cross 29×A would thus be t^0/t^1, that is, the compound of two lethals would be viable. This hypothesis is being tested. Line 19 apparently differs from line A, but there is as yet no evidence (except mode of origin) to indicate that line 19 differs from line 29. No embryological evidence has yet been obtained regarding the assumed lethals in either of these lines.

SUMMARY

Experiments with a pure breeding line of tailless mice discovered by DOBROVOLSKAIA-ZAWADSKAIA show that such tailless mice contain two factors, T and t^0, which are probably alleles, each of which is lethal when homozygous. The combinations of these have the following effects:

Tt^0—tailless;

TT—characteristically abnormal embryos dying about 11 days after fertilization;

t^0t^0—abnormal embryos dying shortly after implantation, probably during the 7th day after fertilization;

T+—short-tailed (Brachyury);

t^0+—normal.

The segregation of t^0 from either T or + occurs normally in oögenesis. When t^0 is transmitted by the male, a marked excess of offspring with t^0 is found, corresponding to a sperm segregation ratio of about 2 t^0:1 +.

Taillessness probably rests on a similar basis in two other tailless lines, that is, the Brachyury lethal T may be balanced by a recessive lethal allele. The second lethal may be different in different tailless lines.

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AN ANALYSIS OF CHROMOSOME STRUCTURE AND BEHAVIOR WITH THE AID OF X-RAY INDUCED REARRANGEMENTS

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THE structure of the chromosome during various phases of the cell cycle is associated with coiling and twisting of the chromonemata. At mitotic anaphase the two chromatids of each chromosome are independently coiled, and remain coiled during the resting stage. At early prophase the minor coils relax and begin to expand. As the relic coils begin to straighten out, new minor coils form in each chromatid. During the prophase stages the two chromatids are twisted about each other or "relationally coiled." Contraction of the chromatids tends to eliminate the relational coiling, so few twists remain at metaphase, and the daughter chromosomes separate freely at anaphase.

The characteristic features of chromosome structure and behavior at meiosis include: (1) the elimination of relic coils *before* new minor coiling is initiated; (2) pairing and relational coiling of homologous chromosomes; and (3) the formation of major coils. The elimination of the relic coils before new minor coiling begins seems to be necessary as a preliminary to chromosome pairing (SAX and SAX 1935). The formation of major coils is not related to chiasma formation or the elimination of relational coiling of chromatids (O'MARA, unpublished). The relational coiling of chromosomes, and perhaps the relational coiling of chromatids in each homologue, seems to be involved in chiasma formation and crossing over (DARLINGTON 1935).

To arrive at an understanding of the mechanism of crossing over, it is essential that as clear a picture as possible be had of each detail of chromosome structure and behavior which is even remotely related to the process of chiasma formation. The relational coiling of chromatids in Tradescantia has been studied directly at second meiotic anaphase, late prophase, and metaphase of the microspore mitotic cycle, when the chromatids are easily seen, and indirectly at the interphase and early prophase by means of chromosome configurations which do not permit free separation of daughter chromosomes. It has been possible to demonstrate what appears to be an increase in the amount of relational coiling during the phase of the mitotic cycle when individual chromatids cannot be completely resolved. The chromosome configurations which make this demonstration possible are of further interest and will be described in some detail.

Microspores of *Tradescantia paludosa* were subjected to approximately 1,000 *r*-units of continuous X-radiation. The normal development of the microspores in this plant has been described in detail by SAX and EDMONDS (1933). The development from the tetrad stage to the division of the microspore nucleus requires about five days during the summer months. The nucleus appears to be in the resting stage for two or three days. During this period the microspore enlarges, and the orientation of the nucleus and cytoplasm is accomplished. The prophase stage begins on the third or fourth day, and the metaphase occurs on the fifth day. The duration of the cycle depends, to some extent, on weather conditions.

The X-ray treatment seems to be more effective in producing abnormality during meiosis than during the somatic resting stage. This differential effect provides a method for timing the duration of microspore development. The young microspores subjected to the X-ray dosage employed continued to develop. The microspores produced *after* X-ray treatment—that is, those which resulted from irradiated meiotic cells—were shriveled and sterile. The microspores which divided on successive days after treatment were X-rayed during the developmental period. When only aborted microspores appeared, it could be concluded that the microspore cycle had ended. The period from treatment to the appearance of only sterile microspores was from 8–9 days, as compared with 5 days for normal plants grown during the summer months. Part of this discrepancy is attributed to greenhouse conditions during the fall and winter months, and part to a retardation of development as a result of treatment.

On the day the treatments were given, and to a lesser degree on the day after, few satisfactory preparations were obtained. The chromosomes were badly clumped and poorly stained. On the second and subsequent days after treatment excellent preparations were possible. Since the cycle from the quartet stage to metaphase was completed in 8–9 days instead of 5, nuclei at metaphase 4 days after treatment were probably rayed during the early prophase stage. Those at metaphase 5–9 days after exposure were undoubtedly resting nuclei at the time the treatment was given. Acetocarmine preparations of the first microspore mitosis, smeared each day after treatment, were made permanent after the method described by BUCK (1935).

OBSERVATIONS

Before the anaphase chromosomes of the second meiotic division enter into the quartet resting stage, they are clearly coiled in the form of minor spirals. It is difficult to determine directly whether the chromosomes at this stage are made up of two spirally wound chromatids "which are not twisted around each other but run parallel as two independent spirals quite

free from each other . . ." as KUWADA and NAKAMURA (1935) report. SAX and SAX (1935) have observed constricted regions in the chromosomes which they interpret as twists in two partially separated spirals, confirming the double nature of the second anaphase chromosomes reported by NEBEL (1932) and KUWADA and NAKAMURA (1935). An analysis of 50 second anaphase chromosomes revealed no constricted regions (half turns which spiralled chromatids make around each other) in 12 chromosomes, 1 constriction in 20 chromosomes, 2 constrictions in 16 chromosomes, and 3 in 2 chromosomes. In 9 of the chromosomes containing 2 constrictions, 1 occurred in each chromosome arm. In the chromosomes with 3 constrictions, 2 were found in 1 arm and 1 in the other. Some of the half turns observed may be cancelled by another half turn in an opposite direction in the same chromosome. Notwithstanding this possibility, a total of 58 half turns were found, or an average of 1.16 half turns per chromosome at second anaphase.[1]

At the earliest prophase, chromosomes are in the form of minor spirals. The minor spirals appear somewhat relaxed, but it is clear that they have not been eliminated during the resting stage. A similar observation has been reported by DARLINGTON (1935), who says: "The correspondence of the spirals seen at early prophase with those seen at telophase shows that no great change of position has taken place during the resting stage—that it is mechanically what its name implies." As prophase advances, the old minor, or relic spirals, appear to relax more completely. They are optically single. The gyres of these spirals tend to increase in diameter and decrease in number as the chromosome shortens. When the presence of relic spirals is no longer obvious, each chromosome is definitely made up of two chromatids which are independently spiralled and tend to be relationally coiled. At least 3 half turns of the chromatids around each other can be seen in some chromosomes at this stage (SAX and SAX 1935).

The direction of the relational coiling in 118 chromosome arms at late prophase and early metaphase was "right" in 60 arms and "left" in 58. The direction in both arms of 50 chromosomes was "right" in 18, "left" in 18; and in 14 chromosomes "right" in one arm and "left" in the other. The direction of relational coiling is apparently not at random in the 2 arms. In approximately a third, instead of a half of the chromosomes, the direction is reversed at the fiber attachment. At late metaphase the majority of the chromatids are parallel or, at most, make an occasional half turn around each other. The relational coiling is evidently reduced as the chromosomes shorten during prophase. In 195 chromosome arms at late

[1] I am indebted to Professor KARL SAX for the loan of the preparation from which these data were taken.

metaphase, 56 half turns (or overlaps) were found. This is an average of 0.57 of a half turn per chromosome. The daughter chromosomes (3 with median and 3 with submedian fiber attachments) measure ca 10–12μ long and 1.3μ in diameter. They separate freely at anaphase.

At the first mitosis after X-ray treatment, daughter chromosomes in three chromosome configurations do not separate regularly in the normal manner. Chromosomes with two attachments, chromosomes with fused chromatids, and ring-shaped chromosomes are configurations of this type. They differ from structurally unchanged chromosomes by usually breaking at the anaphase stage.

Chromosomes with two fiber attachments

Chromosomes with two fiber attachments were found frequently at metaphase of the microspore division. The chromatids between the two attachments were parallel or often twisted about each other. The daughter chromosomes were observed to disjoin in three ways at anaphase.

(1) Both of the attachments on each daughter chromosome go to the same pole. The chromosomes remain intact, and two daughter nuclei result, with the same chromosome situation as the parent nucleus (figs. 1 and 2).

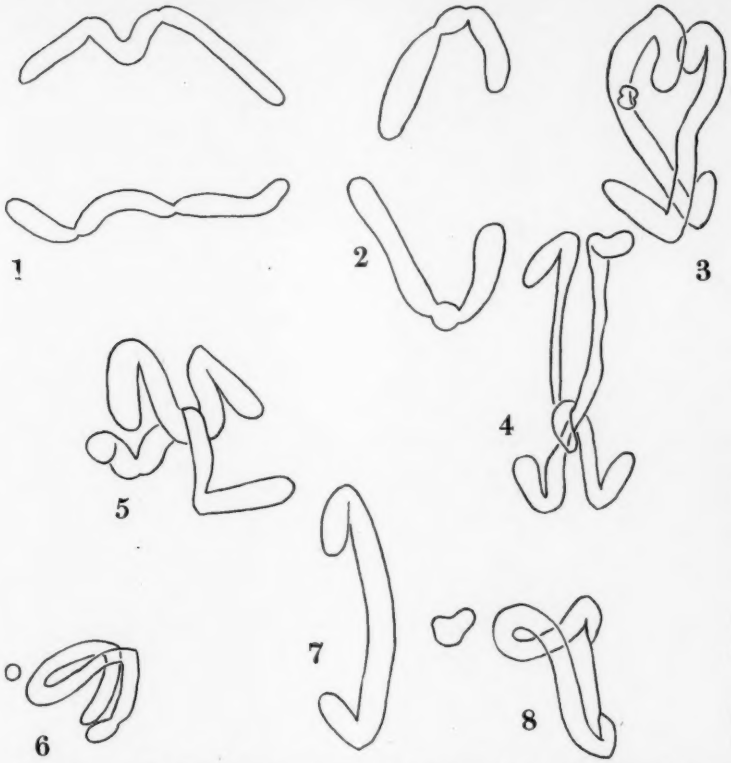
(2) The two attachments on each daughter chromosome go to opposite poles. The chromosomes then become attenuated and are observed to break, as they eventually must if disjunction is to be completed (figs. 3 and 4).

(3) The two attachments on each daughter chromosome go to the same pole but, because the chromosomes are interlocked, at least one and sometimes both chromosomes are broken (fig. 5).

In the cases where the two attachment points on each daughter chromosome pass to the same pole and the daughter chromosomes are interlocked (fig. 5), the two chromatids comprising the segments between attachments have made one complete turn about each other. In figure 3, one half turn is involved; in figure 4, three half turns. In figures 1 and 2, there is no twisting, and the two chromatids must have been lying parallel, in one plane at metaphase.

The number of turns two segments can make around each other is dependent upon the length of the segments and their diameters. If the amount of twisting per unit of chromosome length in one species is to be compared with the amount of twisting in a species with dissimilar chromosomes, both the length and diameter of the chromatids must be considered. The length of the segment between two attachments, divided by the diameter of the daughter chromosome, will give a ratio which can be used to express segment length and to compare twisting.[2]

The ratio was determined for twenty-five chromosomes which disjoin without breaking, for twenty-five which separate as in (2), above, and for twenty-five which separate in a way that necessitates the breakage of at least one daughter chromosome. The averages of these ratios and the extremes in each case are given in table 1. An anaphase chromosome with



TEXT FIGURES 1–8. Mitotic anaphase chromosomes with two fiber attachments and chromosomes with fused chromatids. Figs. 1, 2, and 5. Two attachments on each daughter chromosome moving to the same pole. Figs. 3 and 4. Two attachments on each daughter moving to opposite poles. Fig. 6. Metaphase chromosome with fused chromatids. Figs. 7 and 8. Anaphase chromosomes with fused chromatids. ×2360.

fiber attachments moving toward opposite poles appears approximately twice as frequently as a chromosome which is interlocked.

[2] Unless otherwise stated, the actual length divided by the average diameter of a daughter chromosome and the actual circumference divided by the average diameter of a chromatid or daughter chromosome will be referred to as "length" and "circumference," respectively.

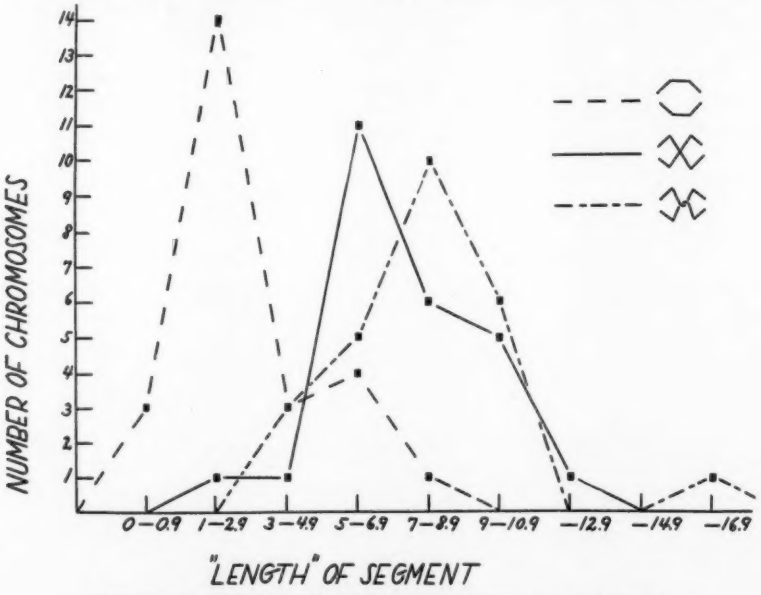
The chromosomes which separate without breaking (1) have an average ratio of 2.5; those which separate in the manner which demands the breakage of both daughter chromosomes (2) have an average of 6.9; and those

TABLE 1

Average "length" of segment between attachments.

Type of disjunction (see text)	(1)	(2)	(3)
Average "length" of segment between attachments	2.5	6.9	7.5
Extremes	0.7–7.0	2.0.11.0	3.0–16
Average "length" of segment in (2) and (3) per 1 full turn		9.6	
Number of chromosomes	25	25	25

which have a breakage of at least one daughter necessary for disjunction (3) an average ratio of 7.5. The results show that at the first division after the formation of a chromosome with two fiber attachments, variation in its behavior can be expected depending upon the "length" of the chromo-



TEXT FIGURE 9. Relation of "length" of segment between two attachments to type of separation at mitotic anaphase.

some segment between the two attachments. If the attachments are relatively close together, they behave usually as a unit, although one may apparently precede the other toward the pole. A chromosome with two attachments widely separated rarely disjoins completely without the breakage of one or both daughters. Seventeen of the 18 chromosomes

which have a ratio under 3 are separating without breaking. Twenty-nine of the 30 chromosomes which have a ratio of 7 or higher are separating in a manner which will result in the breakage of one or both daughter chromosomes (fig. 9).

The three types of disjunction observed can most easily be interpreted as the result of twists present in the sister chromatids when the structural change, resulting in a chromosome with two attachments, was induced. In a normal chromosome these twists tend to straighten out during the contraction of the prophase chromosome, and few are present at metaphase. If chromatids do not rotate at the attachment point, the twists, or turns chromatids make around each other, present between the two attachments of a structurally changed chromosome, could not straighten out. When no turns exist between the attachments, the two attachments on each daughter chromosome pass to the same pole. Should a half turn, one and a half turns, etc., be present, the two attachments on each daughter move to opposite poles. A full turn, two, three turns, etc., necessitate the passage of the two attachments on each daughter to the same pole, and breakage results.

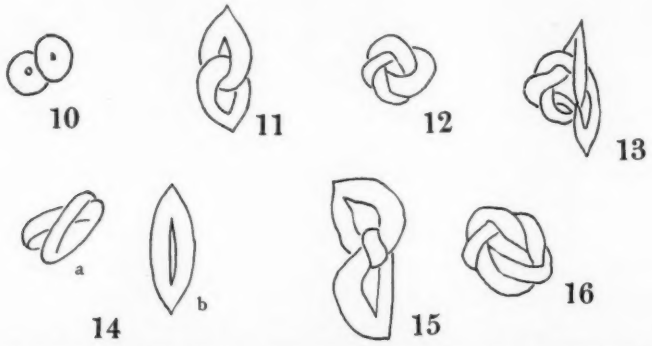
Fused Chromatids

"Chromatin bridges" have been frequently reported as a result of X-ray treatment. These extend from one anaphase group of chromosomes along the long axis of the spindle to the other pole. At the first metaphase after X-ray treatment, chromosomes are often seen which show only two free chromatid ends instead of four. The chromatids in one arm of these chromosomes are so completely fused that it is impossible to determine exactly where a union of ends has occurred (fig. 6). In every cell containing a chromosome with fused chromatids, chromosome fragments are also found. The fused chromatids do not allow free separation of the daughter chromosomes at anaphase. As a result, a chromatin bridge is formed (fig. 7). The attached sister chromatids become attenuated and undoubtedly break. Ring-shaped chromosomes are also found separating at anaphase. The lengths of the two chromatin segments between the sister attachments are of unequal length (fig. 8). These are most probably the result of two chromatid fusions. If the two chromatids of a chromosome with fused chromatids again fuse, after a breakage has occurred at anaphase, and subsequent breakages should occur at points other than at the point of previous union, gene duplication and deficiency would result in this type of chromosome as well as in a chromosome with two attachments.

Ring-Shaped Chromosomes

Sixty-five ring-shaped chromosomes were found at late prophase, metaphase, and anaphase of the first division after treatment. Ten of the 65

were clearly "disjunctional." The two chromatids comprising these rings were not twisted about each other (fig. 10). At anaphase the daughter chromosomes should separate as freely as the chromosomes with two attachments shown in figures 1 and 2. The "disjunctional" rings had an average "circumference" at metaphase of 3.8, the largest a "circumference" of 5. The average "inter-attachment" segment "length" in "disjunctional" chromosomes with 2 attachments (table 1) was nearly 3. Disjunctional rings may be formed by the union of one broken end of each chromatid with its other broken end.



TEXT FIGURES 10–16. Ring-shaped chromosomes at mitotic metaphase and anaphase. Fig. 10. "Disjunctional." Figs. 11–13. "Interlocked" ring-shaped chromosomes. Chromatids make one turn about each other in 11, two turns in 12, and three turns in 13. Figs. 14–16. "Continuous" ring-shaped chromosomes. Chromatids make a half turn about each other in 14 (a, metaphase; b, anaphase), one and a half turns in 15, and two and a half turns in 16. ×2360.

Thirty-seven of the ring-shaped chromosomes were "interlocked." The two chromatids in 31 of them made one complete turn around each other (fig. 11). Twenty-eight of these 31 rings were at metaphase or anaphase, had a circumference no greater than the length of a normal metaphase chromosome, and an average "circumference" of 7.5. The chromosomes with 2 attachments in which the chromatids make one full turn around each other (fig. 5) had an average "inter-attachment" segment "length" of 7.5. In 5-ring chromosomes, the chromatids made 2 full turns around each other (fig. 12), and in one chromosome 3 full turns (fig. 13). The chromosome in figure 13 is at anaphase. It is somewhat stretched and measures ca 16μ in circumference. At metaphase it probably had a circumference no greater than the length of a normal chromosome. These ring-shaped chromosomes range from 3μ in diameter to 16μ in circumference. They may be formed by the union of one broken end of each chromatid with its other

broken end, the sister chromatids being twisted 1–3 times around each other prior to the union.

Eighteen of the ring-shaped chromosomes were "continuous." In 14 of them, the two chromatids made a half turn around each other (fig. 14). The 10 simple "continuous" rings which were at metaphase or anaphase, could be measured and had a circumference no greater than the length of a normal chromosome, had an average "circumference" of 6.5. The chromosomes with two attachments in which the chromatids made a half turn around each other had an average "inter-attachment" segment "length" of 6.9. In 3 of the ring chromosomes the chromatids made 1.5 turns around each other (fig. 15); and in one, 2.5 (fig. 16). The "continuous" rings may be formed by the union of one broken end of each chromatid with the other broken end *of its sister*, 0–2 *additional* full turns of the sister chromatids about each other being involved. The 38 simple "interlocked" and "continuous" ring chromosomes measured had an average "circumference" of 8.3 per full turn.

At anaphase the "interlocked" rings cannot disjoin without the breakage of at least one daughter chromosome. The simple "continuous" ring opens out after metaphase to form a chromosome twice the size of the original configuration. This double-sized ring, as well as the more complex "continuous" rings, must break in two places during the anaphase separation.

There does not appear to be a statistically significant difference in the average "circumference" of the simple "interlocked" and "continuous" ring-shaped chromosomes produced by X-ray treatment of the early resting, the late resting, or the early prophase stages (table 2). Five of the more complex ring-shaped chromosomes (that is, those in which the

TABLE 2

Average "circumference" of ring-shaped chromosomes.

Number of days between x-ray treatment and metaphase	2–4	5	6–9	2–9
Approximate stage treated	early prophase	late resting	resting	—
Average "circumference" of simple interlocked rings	7.3±1.01*	6.98±.49	8.64±.75	7.47
Number of chromosomes	7	14	7	28
Average "circumference" of simple continuous rings	—	6.6±1.12	6.46±1.16	6.53
Number of chromosomes	—	5	5	10
Average "circumference" of simple continuous and interlocked rings	7.3	6.87	7.73	7.23
Total number of chromosomes	7	19	12	38

* $s^2=\frac{\Sigma(x^2)}{N-1}$; $S_{\bar{x}}=\frac{s}{\sqrt{N}}$

chromatids make more than 1 full turn around each other) resulted from treatment applied at the resting stage, and five from treatment given at early prophase.

DISCUSSION

A normal metaphase chromosome at the first microspore mitosis in Tradescantia has a "length" of ca 4–5 for each arm. The chromatids of this chromosome are usually lying parallel, or at most make a half turn around each other. An average of ca 2 half turns for every 7 arms, or 0.57 of a half turn per chromosome, is present at this stage. In figure 9, 7 half turns are found between the 2 attachments in the 7 segments with "lengths" of 3–4.9. The 50 chromosomes with 2 attachments, between which $\frac{1}{2}$ or 1 full turn occurs, have an average "length" per 1 full turn of 9.6 (table 1). The 38 ring-shaped chromosomes, in which $\frac{1}{2}$ or 1 full turn of the chromatids around each other is present, have an average "circumference" per 1 full turn of 8.3. The number of turns per unit of "length" and "circumference" in chromosomes with 2 attachments and ring-shaped chromosomes is approximately equal and over three times the average number of turns found in a normal metaphase chromosome.

There may be at least 1.5 full turns of the chromatids around each other in a normal chromosome at late prophase. At metaphase this number has been reduced, so at most an occasional half turn or overlap is present. A reduction in the amount of relational coiling is obviously prevented in ring-shaped chromosomes and chromosomes with two attachments. In ring-shaped chromosomes there are no free ends to rotate. Any relational coiling present at the time these chromosomes were formed could not be eliminated by the contraction of chromosomes during prophase and early metaphase. Since the amount of relational coiling in ring-shaped chromosomes and chromosomes with two attachments is approximately equal at metaphase, an interesting property of the fiber attachment is demonstrated by means of these configurations.

The fiber attachment has been known for some time as the constant and well differentiated portion of a chromosome which separates first at anaphase and precedes the body of the chromosome toward the pole. In many organisms the chromatids are so intimately associated at the fiber attachment that this limited section of the chromosome appears to be single. At late metaphase, after the chromatids have become oriented, the anaphase separation is first initiated at the region of the chromosome which previously showed no evidence of being two-parted. The character of the fiber attachment region apparently changes abruptly during metaphase. The change, according to some workers, is probably brought about by its division at this stage. The fiber attachment is known to be four-parted

at metaphase of the microspore division, and two-parted at anaphase in chromosomes which are especially favorable material for the study of chromosome structure (Trillium; HUSKINS and HUNTER 1935). A half turn of sister chromatids around each other may be present in a segment between two attachments which is no longer than twice the diameter of a daughter chromosome (fig. 9). The contraction of the chromosome does not produce a strain which is great enough to cause a rotation of the chromatids at the fiber attachment. Although this portion of the chromosome is 2-parted, it behaves essentially like an undivided region prior to anaphase.

The direction of relational coiling in the arms of a Tradescantia chromosome does not appear to be at random. It changes at the fiber attachment in only about a third of the chromosomes. If, in the formation of a ring-shaped chromosome, breakage occurs at the time of treatment, and the union of broken ends is accomplished immediately, there would be no opportunity for an untwisting of chromatids that are relationally coiled in the same direction on both sides of the attachment, between the time of treatment and metaphase. The average number of turns sister chromatids make around each other per unit of chromosome "circumference" at metaphase would be a rough index of the number present at the time of treatment. If, on the other hand, breakage occurs at the time of treatment and union is delayed, the amount of relational coiling in ring-shaped chromosomes would be an indication of the average amount present when the union of broken ends is accomplished. This union may not take place until chromosome contraction at prophase has eliminated some of the relational coiling. The chromatids of the ring-shaped chromosomes (and chromosomes with two attachments) average 2 half turns around each other in a "circumference" (or "length") which is slightly less than the "length" of a normal metaphase chromosome. It seems evident that the chromatids of such chromosomes averaged at least 2 half turns around each other at the time the broken ends united. In one ring-shaped chromosome 6 half turns were found. Approximately a third of the ring-shaped chromosomes were continuous, and no "disjunctional" ring had a "circumference" greater than half the "length" of a normal chromosome.

The two chromatids of a second anaphase chromosome in Tradescantia may average 1.16 half turns around each other (maximum number of half turns found in one chromosome is 3. After the formation of a ring-shaped chromosome, a half turn of chromatids around each other on one side of the attachments would be cancelled by a half turn in the opposite direction on the other side. When it is considered that the direction of relational coiling in the two arms of a second anaphase chromosome may be at random, or may change at the fiber attachment in about a third of the chromo-

somes (as it does at prophase), the average amount of relational coiling at anaphase, which would be expected to persist in a ring-shaped chromosome, becomes nearer one half turn per chromosome. The ring-shaped chromosomes (as well as the chromosomes with two attachments) seem to indicate that the amount of relational coiling is increased to an average of at least two half turns per chromosome (maximum number of half turns in one ring-shaped chromosome is 6) some time before late prophase of the next mitotic division. The maximum amount of relational coiling seen in the ring-shaped chromosomes is twice the maximum amount in a normal chromosome at the preceding anaphase. In structurally unchanged chromosomes, the average amount is reduced, as a result of chromosome contraction during prophase to 0.57 of a half turn which remains at metaphase.

McCLINTOCK (1932) has shown that broken ends of chromosomes have a strong tendency to unite with broken ends. Whether the union immediately follows breakage or is possibly delayed, even for some cell generations, has not been definitely determined (STADLER 1932). The similarity of the results of X-ray treatment applied during the early resting, late resting, and early prophase stages may be brought about by the delay of the union of broken ends until one prophase period, regardless of the stage at which the chromosomes were broken, or by a union concomitant with breakage. If the union of broken ends immediately follows breakage, an increase in the relational coiling of chromatids must occur during the telophase which precedes the resting and early prophase stages exposed to X-rays. If the chromosome ends, broken at different periods of the resting and early prophase stages, unite at a given midprophase period, an increase in relational coiling could take place at the beginning of prophase rather than at the preceding telophase.

A brief consideration of mitosis and meiosis may suggest which of these explanations is the more tenable, and at what stage of the somatic cell cycle an increase in relational coiling might be brought about. The two chromatids of a mitotic anaphase chromosome seem to be in the form of tightly intermeshed (not interlocked) minor spirals (NEBEL 1932; KUWADA and NAKAMURA 1935). They do not appear to be twisted around each other to any great extent. During the resting stage the spirals become relaxed, but they are not eliminated (DARLINGTON 1935; SAX and SAX 1935). As the nucleus enters mitotic prophase and enlarges, the gyres of each relic spiral become wider, and their number seems to be reduced as prophase advances and the chromosomes become shortened. When the spirals are nearly straightened out, each chromatid is seen to be independently spiralled, and tends to be relationally coiled. The new spirals further shorten the chromatids during later prophase. The twists are passed off the ends of the chromosome, so few remain at metaphase.

The increase in size of the early meiotic nucleus and the leisurely progress of the meiotic prophase permit a complete removal of the relic spirals before the new spiralization begins. At pachytene the chromosomes are extended to approximately twice the length seen at a comparable mitotic prophase (SAX and SAX 1935). The mitotic and premeiotic resting stages appear to be identical. The essential difference between a mitotic prophase and an early meiotic prophase is the period at which the new spiralization begins in relation to relic spiral elimination. In mitotic prophase spiralization of chromatids begins at early prophase, before the relic spirals of anaphase are drawn out. When the relic spirals of the somatic chromosome are eliminated, the new minor spirals are well formed and the chromosome has been shortened to half its expanded length as seen in a meiotic chromosome at pachytene. Relational coiling of chromatids is easily reduced as a chromosome shortens during late mitotic prophase, and becomes more rigid as the formation of minor spirals is completed. In meiotic prophase the chromosome elongates and "pairs" with its homologous chromosome. The elimination of the relic spirals (which is probably the major factor in the mechanism of elongation) is not accompanied by the formation of new minor spirals. The formation of these spirals is not initiated until *after* homologous chromosomes have become intimately "paired" (SAX and SAX 1935).

A modification of the torsion theory of crossing over, proposed by WILSON and MORGAN (1920), seems to be most in accord with the cytological observation of bivalent chromosomes. This theory demands that the chromatids in each chromosome which associates with its homologue be parallel and *not twisted around each other* (SAX 1936). If this theory is sound, and if in nature there is an actual increase in the amount of relational coiling of chromatids at the telophase stage of mitosis, this coiling would have to be eliminated before the association of homologous chromosomes at meiotic prophase occurs. At early meiotic prophase the chromosomes are elongating and presumably becoming more flaccid. These chromosomes would more probably retain any relational coiling present than remove it. If an increase in the amount of relational coiling actually occurs when new minor spirals are being formed (that is, at early prophase of mitosis and late pachytene of meiosis) the requirement of the torsion theory, in regard to relational coiling of chromatids, could be fulfilled. It seems more probable at present that an increase in relational coiling of chromatids is initiated at early prophase in mitosis when the formation of new minor spirals begins; and that the union of ends broken at the resting and early prophase stages is not definitely accomplished until a later prophase period.

How should the chromosomes with two attachments and the ring-shaped

chromosomes described at the first mitosis after their formation be expected to behave during later somatic divisions? MATHER and STONE (1933) have reported the occurrence of chromosomes with two attachments in X-rayed Crocus and Tulipa root tips. They point out two ways in which these chromosomes can separate: the two attachments on one daughter chromosome can go to the same or to opposite poles. If they move to opposite poles, the chromosomes become attenuated and probably break, eventually giving rise to daughter cells which have chromosomes with only one attachment and which differ from the parent nucleus. It is reasoned, since the chances of either mode of division are taken to be equal, that the probability of a chromosome with two fiber attachments surviving after n cell generations is 2^{-n}; that "there is . . . a strong tendency for such chromosomes to disappear. . . ." This seemed to be the case in Crocus. Fixations made soon after treatment showed "a few" chromosomes with two attachments, while later fixations showed none at all. In Tulipa, however, four chromosomes with two attachments (two of the four existing together in a single nucleus) were found in root tips fixed three months after treatment.

These chromosomes, illustrated in MATHER and STONE's figure 32, have ratios of ca 5, 11, 13, and 14. If the original chromosomes from which these four descended had like ratios, and it is assumed that Tulipa chromosomes with two attachments behave, at the first division after their formation, like Tradescantia chromosomes, only one of the four could have disjoined without a breakage.

If fifty can be taken as a fair estimate of the number of cell generations accomplished during a period of three months and the chances of either mode of division of a chromosome with two attachments are equal, the probability of such a chromosome surviving after fifty cell generations would be 2^{-50}. The finding of four of these chromosomes suggests that the chances of either mode of division are unequal, or, when breakage occurs, the broken ends tend to unite like the broken ends of ring-shaped chromosomes.

MCCLINTOCK (1932) and MCCLINTOCK and RHOADES (1935) have presented the most complete study of ring-shaped chromosomes and their behavior. These chromosomes do not descend through many cell generations unaltered. Losses, and less frequently additions, of sections of chromatin are continually occurring. These changes, they have suggested, may be causally related to the way a ring chromosome becomes split during mitosis. The product of chromosome splitting is not always two ring chromosomes lying side by side. "Instead, especially with large rings, many anaphase figures reveal that the end product of the splitting process has produced two interlocking rings or one large double-sized ring with two

spindle fiber attachment regions. In this latter case, the large single ring with the two spindle fiber attachment regions is built up from both split halves of the mother ring. On the simple hypothesis that the chromosome may start its splitting process at more than one place in the chromosome and that the planes of the splits so started do not correspond, it is relatively easy to see how such figures could be obtained. In both the interlocking and double-sized rings, the pull on the chromosomes produced by the passage of the spindle fiber attachment regions to opposite poles introduces a strain on the chromosomes which eventually leads to breakage. In the case of the double-sized ring with the two spindle fiber attachment regions, the chromosome does not always break in the middle but may break in several places, the parts adjacent to the spindle fiber attachment region at both poles passing into the telophase nuclei, the other parts being left in the cytoplasm. Obviously, then, the chromatin content and structural arrangement of the original ring has become altered. It might be expected that this mechanism would produce rod-shaped chromosomes. On the contrary, the broken ends thus produced apparently reunite to form rings."

The assumption of the occurrence of a chromosome split in more than one plane is necessary to explain the behavior of persisting ring-shaped chromosomes that are continually breaking if the chromonema is single at mitotic anaphase, and the "permanent" union of broken ends is accomplished before any relational coiling of chromatids can occur. In Tradescantia, and in other plants with large chromosomes, there can be no doubt that the mitotic telophase chromosomes are at least two-parted. There is considerable evidence that they are four-parted (NEBEL 1932; STEBBINS 1935; GOODSPEED, UBER and AVERY 1935). The anaphase chromosomes of the second meiotic division in Tradescantia are two-parted (NEBEL 1932; KUWADA and NAKAMURA 1935; SAX and SAX 1935; DERMEN 1936). All the ring-shaped chromosomes which were found at the first division of the microspore in Tradescantia were formed, therefore, after the chromosome split had occurred. One or both daughter chromosomes resulting from the majority of these rings will break at anaphase. At the time the breakage occurs, the daughter chromosomes will be at least two-parted, as was the parent ring at the time of its formation. A broken end of an anaphase chromatid may unite as often with the broken end of its sister as with its other broken end. Ring chromosomes which are continually breaking might persist in this way. Whenever the two broken ends of one chromatid unite, however, and the two sister strands are *not twisted*, a "disjunctional" ring-shaped chromosome would result. There would be a trend toward replacement of the "continuous" and "interlocked" types by "disjunctional" rings which separate without breaking unless relational

coiling of chromatids is increased before each union of broken ends. If the complete union of broken ends is delayed until somatic prophase, until after a relational coiling of chromatids has taken place, the behavior of ring-shaped chromosomes which are persistently breaking becomes understandable without assuming that the chromosome split occurs in more than one plane. The chromatids of these chromosomes, especially the larger ones, may become relationally coiled and the broken ends fused completely at prophase after each breakage that is brought about during the previous anaphase separation. Chromosomes with two attachments should behave in the same manner.

SUMMARY

During the mitotic cycle the two chromatids of each chromosome are twisted about each other or relationally coiled. The amount of relational coiling during the preceding anaphase and late prophase and metaphase in microspore development of Tradescantia has been studied directly. During the resting stage, and early prophase, direct analysis is impossible, so these stages have been studied by means of X-ray induced chromosome configurations which retain their relational coiling until they break at anaphase.

Among the abnormalities obtained after X-ray treatment were chromosomes with two fiber attachments and ring-shaped chromosomes. The amount of relational coiling was approximately the same in both of the configurations. A study of these chromosomes shows that the reduction of relational coiling in a normal chromosome does not involve the rotation of chromatids at the fiber attachment point. The proportions of free and locked associations differ in the two types and can be attributed to the fact that the direction of relational coiling is at random for any two chromosomes, but tends to be in the same direction in the two arms of a single chromosome which fuse to form rings.

The amount of relational coiling at metaphase in the ring-shaped chromosomes appears to be the same, regardless of the time of X-ray treatment between telophase and early prophase. This evidence suggests that the union of broken ends of chromosomes is not effected at the time of X-ray treatment, but occurs at a certain stage in prophase development. Such behavior would explain the persistence of ring-shaped chromosomes which are continually breaking.

The available evidence indicates that there is little relational coiling of chromatids at anaphase during mitosis, an increase during prophase as new minor spirals are formed, and a decrease during very late prophase and metaphase as each chromatid becomes shortened, more rigid, and the formation of minor spirals is completed. In the meiotic prophase relational coiling is not initiated until homologous chromosomes are paired.

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THE RELATIONS OF INVERSIONS IN THE X CHROMOSOME OF DROSOPHILA MELANOGASTER TO CROSSING OVER AND DISJUNCTION*

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INTRODUCTION

STUDIES of chromosome aberrations such as polyploidy and translocations have contributed much to the understanding of the meiotic behavior of chromosomes. One of the commonest types of structural difference in chromosomes within a species is that in which a segment of a chromosome has been inverted. These cases have not contributed as much as might have been expected toward an understanding of chromosome mechanics. It has been apparent for some time that they needed systematic study, and that the series of X chromosome inversions accumulated in X-ray experiments furnished the necessary material. The present paper represents the results of a study made with these points in mind.

* The cost of the accompanying tables and illustrations is paid by the Galton and Mendel Memorial Fund.

Recent papers on inversions illustrate the difficulties encountered in such studies. GERSHENSON (1935) and STONE and THOMAS (1935) found that the chromatids resulting from single crossing over are not recovered, and by making egg counts showed that there is no detectable mortality that can be invoked to account for them. They concluded that single exchange is so rare as to be negligible. SIDOROV, SOKOLOV, and TROFIMOV (1935) showed by the use of attached-X females that single exchanges do occur with a high frequency; but they appear to have been unaware that egg counts fail to show any corresponding mortality. The dates show that at least the second and third papers mentioned and our own preliminary note (BEADLE and STURTEVANT, 1935) were all sent to press inde-

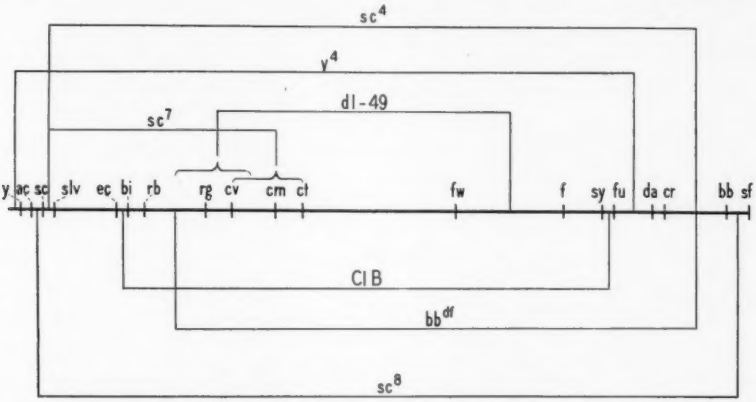

FIGURE 1.—Diagram showing the nature of the inversions used in the present study.

pendently. Our account, of which the present paper is the full presentation, included both the proof that single exchanges occur (using the same method as the Russian investigators), and the proof that there is no corresponding egg mortality. We were faced with a seeming paradox, the only escape from which was the assumption that the single crossover chromatids are produced but are not included in the egg nucleus. We developed a scheme that gives this result (pp. 591–596, and figs. 6 and 7), and that is in good quantitative agreement with the data in other respects.

INVERSIONS STUDIED

There follows a descriptive catalogue of the inversions we have studied. Figure 1 shows the lengths of these inversions. The data on the extent of each inversion are largely from the experiments with females heterozygous for two inversions, which are described below. The egg mortalities associated with the inversions are given in a later section.

Inversion scute-4

AGOL (1929) subjected yellow flies to X-rays, and obtained an extreme scute allelomorph, *sc*^{4}. This was found to be associated with a long inversion in the X chromosome, shown below to extend from a point between *sc* and *slv* to a point between *cr* and *bb*. Females heterozygous for this inversion give about 9 percent crossing over among their regular offspring, and also produce about 6 percent of patroclinous sons, as shown in table 1. All the recovered crossovers are doubles.

Our best experiments are those in which *y*, *cv*, *v*, and *f* are followed, since here it is probable that few or no undetected doubles occur. The table shows a few crossovers entered as singles; these are evidently all really doubles in which the second crossover has occurred in the unmarked region between *f* and the inversion point. In another experiment, in which *cr* and *bb* were present, but the rest of the chromosome was not so well controlled, two such apparent singles were tested and found in fact to represent *cr bb* crossovers as well.

Table 1 shows a total of 108 crossover males to 63 patroclinous from XX females; the ratio between these classes, 1.71:1, will be discussed below.

TABLE 1

Tests of females heterozygous for inversion sc-4. In this and following tables the crossover classes are labelled according to the standard sequence of loci, unless otherwise indicated. In cases where two contrary classes are entered under one heading, the right-hand or lower one of the two carries the mutant gene at the leftmost locus concerned.

MOTHERS	♀		REGULAR ♂♂														EXC. ♂♂
	REG.	EXC.	0		1		2		3		1, 2		1, 3		2, 3		TOTAL
sc^3 *cv v f*/*y sc*^{4}	344	0	115	150	1	2	2	1	1	0	1	0	3	5	4	6	291	18
y^2 *cv v f*/*y sc*^{4}	331	0	154	86	3	0	0	0	1	2	1	0	4	7	5	3	266	14
v/*y sc*^{4} *cv f*	801	1	294	291	2	3	7	6	0	2	3	2	10	11	6	4	641	31
y^2 *cv v f*/*y sc*^{4}/*Y*	397	11	144	105	3	1	4	3	1	4	3	2	9	9	4	7	299	52

The total crossover percentage among regular sons is 9.3 from the XX females, 16.7 from the XXY females. The latter value needs correction, however. Half of the exceptional gametes die, so the totals should be taken as regulars plus twice the exceptional females; this gives 50:299+22, or 15.6 percent. It seems clear that the presence of a Y significantly increases the frequency of recovered double crossovers.

Crossing over in sc-4/sc-4 is approximately normal. Three experiments are recorded (table 2). The third experiment also gave a total of 14 reversions of Bar and 6 occurrences of double-bar (in 8,523 flies), all of them *f-fu* crossovers. In many of these cultures the *B* flies were not classified

for *f-fu*. This serves to confirm the report of MULLER and WEINSTEIN (1933), based on sc-8 experiments, that unequal crossing over occurs only between non-sister strands even when the *B* locus is far removed from the spindle attachment.

TABLE 2

Crossing over in homozygous inversion sc-4. Regular males only are recorded in the first experiment; in the second and third both males and females are included.

MOTHERS	0		1		2		3		1,2		1,3		2,3		TOTAL
cv cr/w^e f	66	73	14	8	43	42	9	16	2	3	1	1	1	2	281
vf/cv	93	94	31	20	24	37			3	0					302
B/f B fu	2707		103												2810

Inversion scute-7

DUBININ (1930) has described scute-7. It was obtained by X-raying apricot (w^a) flies, and is a recessive scute allelomorph resembling but definitely different from scute-1.

Females heterozygous for scute-7 have shown no crossing over for any loci to the left of *ct*, and a reduction of crossing over for the interval from *ct* to *lz*. To the right of *lz* substantially normal values have been obtained (table 3). Tests of crossovers have shown that the decrease of crossing over is due to something lying to the left of *sn*, in the region most affected.

TABLE 3*

LOCI	sc-7/+		sc-7/sc-7	
	N	PERCENT	N	PERCENT
sc-w	6213	0	—	—
w-cv	3103	0	—	—
fa-sn	—	(0.4)	603	2.8
cv-ct	1030	0	—	—
cv-sn	1395	0.4	—	—
cv-v	938	7.5	—	—
sn-lz	1395	1.6	292	5.1
lz-v	—	(5.5)	292	3.4
v-f	678	24.9	350	22.6

* These values are all from XX females. Less extensive data are available for XXY females in the case of sc-7/+; they show no significant differences from the above values.

Owing to the complete absence of crossing over to the left of *ct* it was at first impossible to test this region in homozygous sc-7. However, in a sc-7 chromosome a mutation occurred to an allelomorph of facet, found in an X-ray experiment by Mrs. C. E. RUCH. The results obtained with this chromosome, tested against genes introduced by crossing over (table 3), show that the reduction of crossing over in heterozygous sc-7 is due to an inversion that includes *fa* but not *sn*.

The extent of this inversion has not been determined accurately by genetic methods, since it does not give viable crossovers with any other inversion we have used, unless special methods are used. Salivary gland chromosomes have, however, been seen to give typical heterozygous inversion figures that show the existence of a short terminal uninverted piece. Dr. J. SCHULTZ has studied such preparations more carefully, and we are indebted to him for the information that the left inversion point lies close to the right of scute, the right one not far from the locus of crossveinless.

Inversion scute-8

SIDOROV (1930) subjected apricot (w^a) flies to X-rays and obtained a bristle mutant described as a new scute allelomorph. The relations of this to the scute and achaete series are peculiar; but it is most convenient to retain the name scute-8 for it. There is an associated inversion which has been studied by several investigators (PATTERSON and STONE 1935; STONE and THOMAS 1935). These authors have described its properties; the data given here are in essential agreement with their account.

As will be shown below, the inversion extends from a point between *ac* and *sc* to a point to the right of *bb*. Its right end has been shown by GERSHENSON to be located in the inert region of the X.

The crossing over shown by sc-8/+ is illustrated in table 4. Our other experiments are less satisfactory in that fewer or less well-spaced loci are concerned, but they agree in indicating that these are about the usual values. As in other cases, the classes listed as single crossovers are obviously really doubles, with the second crossover between forked and the right inversion point.

No adequate data are available for crossing over in XXY sc-8/+ females.

Table 4 shows that crossing over is of about the normal frequency in sc-8/sc-8, though (as briefly stated by OFFERMAN and MULLER, 1932) there are local differences from the standard values. The w^a-*cv* interval gives 6.1 percent, as opposed to the usual value of about 12 percent, whereas *f-cr* gives 9.5 as opposed to about 6 percent. Thus in both cases the same section gives more crossing over when it lies far from the spindle attachment.

Inversion scute-8 deficiency

The scute-8 inversion reaches from a point between *ac* and *sc* to a point to the right of *bb*. We have found, as NOUJDIN (1935) has recently reported, that any series of sc-8, in which appropriate tests are made, produces occasional *y-ac* deficiencies, that is the left inversion point involves an unstable union of parts. The resulting chromosome is deficient for *y*, *ac*, and probably *Hw*, and for no other known loci. All the remaining known

loci are in reverse sequence. We have used this "Df sc-8" in some of our inversion experiments.

TABLE 4

Crossing over in inversion sc-8 females.

CLASSES	In/+		In/In			
	sc-8 w^a cv v f	ct	w^a ct cr	+	w^a cv	w^a v
	y^2	sc-8 w^a cr	cv f	w^a cv v f	v f	cv f
0	764	341	490	407	173	78
	913	277	509	257	123	79
1	0	1	33	26	13	9
	0	1	47	31	11	5
2	4	1	29	97	52	18
	3	4	42	75	27	21
3	9		345	110	54	25
	14		326	94	43	20
4	0		74			
	4		71			
1, 2	0	6	0	1	0	0
	0	14	1	1	1	0
1, 3	0		13	3	4	4
	0		13	5	2	4
1, 4	0		6			
	1		9			
2, 3	2		9	18	10	3
	3		10	5	2	1
2, 4	8		6			
	12		2			
3, 4	20		12			
	13		15			
1, 3, 4	0		0			
	0		1			
2, 3, 4	0		0			
	0		1			
Exc. males	57	21				

As in the cases of sc-4/+ and sc-8/+, the apparent single crossovers are evidently all really doubles, in which the second crossover was to the right of *f*. The relative numbers of double crossover males (30) and patroclinous males (31) need correction for comparison with other series, since only half the crossovers survive while the other half carry the deficiency. As thus corrected the ratio becomes 60:31 = 1.9:1.

Tables 4 and 5 indicate that there are about twice as many crossovers recovered from Df (sc-8)/+ as from sc-8/+. Analysis of a few other crosses,

TABLE 5*

$$\frac{sc\ cv\ v\ f}{\text{Df (sc-8) } w^a} \times y^2\ cv\ v\ f$$

INTERVAL	MALES	FEMALES	
0	211	245	245
1	2	4	5
2	2		
3	7	16	9
4	1	1	6
1, 3	0	1	1
2, 3	0		
1, 4	1	2	5
2, 4	7		
3, 4	10	8	7
Total	241	555	
Ex.	31		

* Df sc-8 is lethal, so that each crossover class of males is represented only once. The females could not be classified for w^a, so intervals 1 and 2 were not separable in them. In the female row the not-yellow class is entered to the left in each case. Egg counts made by Miss M. Groscurth show that the males carrying Df sc-8 die in the egg stage.

not here reported in detail because they include fewer genes or for similar reasons, suggests that the difference, if present, may be less than indicated. More experiments are needed.

Females of the constitution Df (sc-8)/sc-8 have all their genes in the same sequence, but are heterozygous for a terminal *y-ac* deficiency. Table 6 gives the crossing over observed.

TABLE 6

Crossing over in sc^8B/Df (sc-8) *cv v f* ♀♀.*

0		1		2		3		4		1.2		1.3		2.3		TOTAL
89	62	26	16	8	6	2	5	0	0	29	2	25	27	7	9	332

* This table gives the results of a cross to *y cv v f*; it includes both sexes, and contrary classes are therefore not equivalent because of the lethal nature of the deficiency. In reality the sequence is Df *B f v cv*; the classes above, renumbered on this basis, become, in the order given: 0; 1; 1.4; 1.3; 1.2; 4; 3; 3.4).

From table 6 values for comparison with sc-8/sc-8, have been calculated:

	w^a-*cv*	*cv-ct*	*cv-v*	*v-f*	*f-B*	*f-cr*	*B*-Df
sc-8/sc-8	6.1	4.8	17.4	21.3	—	9.5	—
sc-8/Df (sc-8)	—	—	24.1	22.6	0	—	19.0

In the case of *cv v* the data suggest a difference; other experiments (involving fewer loci) with sc-8/Df (sc-8) give values of 18.7 (in 215 flies) and 16.6 (in 380 flies). Both series are therefore to be taken as giving not far from 18 percent.

Inversion ClB

The "ClB" chromosome was first briefly described by MULLER (1928), and has been very extensively used since in special experiments. The most detailed account of its properties is that given by GERSHENSON (1935). PAINTER (1934) has figured the inversion that is visible in the salivary-gland cells of ClB heterozygotes. As described by MULLER, this chromosome has an inversion (shown below to extend from a point between *ec* and *bi* to a point between *sy* and *fu*), and carries a lethal and the mutant genes *sc*, *t*, *v*, *se*, and *B*. The leftmost of these, *sc*, lies outside the inversion and separates from it by crossing over occasionally. The other mutant genes lying in the inversion are also occasionally lost by double crossing over. We should interpret the "reinverted" ClB chromosome described by GERSHENSON (1930) as having arisen from a triple crossover—a double within the inversion and a simultaneous single to the left of it. GERSHENSON (1935) records a total of 1 percent crossing over for the whole chromosome (*sc* to *cr*), a value that is, if anything, slightly higher than general experience would lead one to expect. About three-fourths of this crossing over is made up of singles to the left of the inversion. Our own data are less extensive than GERSHENSON's, and need not be presented since they add nothing of importance.

GERSHENSON has also studied crossing over in XXY females heterozygous for ClB. He found, as have we, that the exceptional females produced are practically always non-crossovers. After correcting his observed crossover values by adding the non-disjunctional eggs to the observed non-crossover regulars, he arrived at the conclusion that approximately the same amount of crossing over occurs as in XX females. His values are 1.29 percent for crossing over to the left of the inversion, .07 percent for doubles within the inversion, the differences from the XX values being opposite in sign and insignificant in amount; the percentages are, however, too small to be useful for such comparisons.

GERSHENSON has added one other essential bit of evidence, concerning a relation we have not studied. He determined (the method used is not stated) the frequency of XX and XXY females among the regular daugh-

ters of XXY ClB females. His table IV shows that among the regulars carrying the ClB chromosome there were 47 XXY:49 XX; among those carrying the non-inverted chromosome there were 68 XXY:63 XX. Evidently, in the regular eggs the Y is distributed at random.

A similar relation was recorded by BRIDGES (1916) for XXY females carrying no inversion, half the regular daughters being shown to be XXY, the other half XX. KIKKAWA (1932) reports that in *D. virilis*, significantly less than half are XXY. This result is based on cytological observations and seems to us to be doubtful. The Y of *virilis* is not visibly different from the X's or from eight of the autosomes; under these circumstances wholesale counts made on the minute oögonial chromosomes seem questionable.

TABLE 7*

$$\text{♀}\ \frac{\text{dl-49}\ cm^2\quad bb}{v\ cr} \times \text{♂}\ v\ B\ bb$$

	XX ♀♀		XXY ♀♀	
Regular (*B*) ♀				
0	220	201	134	255
1	30	40	152	57
Exc. (+) ♀	0		578	
Reg. (not-*B*) ♂				
cm or *cr* (*v* or not-*v*)	419		567	
v	19		40	
Exc. (*v B*) ♂	1		480	

* In the male classes *bb* cannot be identified, and *cm* and *cr* are not separable. The *v-cr* crossing over can only be determined by doubling the *v* class of males, the contrary crossover, *cm cr*, not being identifiable. In the female classes the crossing over detected is that between *v* and *bb*; in the case of the XXY females *bb* is suppressed by the Y present in some of the regular daughters so the observed classes are misleading. Using *bb* classes only the numbers are: non-crossovers 134, crossovers 57.

Inversion delta-49

Inversion dl-49 was first described in an abstract by MULLER and STONE (1931). The left break was shown to lie between *rb* and *cm*, the right between *fw* and *f*. According to PAINTER (1934) unpublished data of these workers show that the right break lies between *fw* and *g*. PAINTER (1934) showed from a study of salivary chromosomes of females heterozygous for inversion dl-49 that the left break occurred between *rb* and *cv*. Since the method that we have used for determining the ends of inversions could not (with the material at our disposal) be used with dl-49, we can add no further information.

Females heterozygous for Inversion dl-49 give few or no crossovers within the inversion among their offspring. We have not collected any ex-

tensive series of data showing this to be true since it seemed quite unnecessary. Inversion dl-49 is extensively used in "balanced" stocks and, so far as we know, no case of double crossing over within the inverted segment has occurred in heterozygous females; the opportunities for detecting such, had they occurred, have been abundant.

Females homozygous for inversion dl-49 show approximately normal crossing over both within the inversion and outside it, according to STONE and THOMAS (1935).

Females heterozygous for inversion dl-49 do give recoverable crossovers outside the limits of the inversion, those to the left occurring with considerably lower frequency than those to the right. Among 533 progeny of the cross inversion dl-49 *cm*^{2} *f*/*y ec cv ct*^{6} *v g*^{2} *f* × *y ec cv ct*^{6} *v g*^{2} *f*, 3 or 0.56 percent were crossovers between *y* and *ec*.

The data of table 7 show, from XX females, *v-cr* = 8.7 percent, *v-bb* = 14.3 percent. The latter value may be taken as giving the amount of crossing over between the inversion and the spindle attachment; it is in reasonable agreement with the value of 13.4 obtained by SCHULTZ (quoted by L. V. MORGAN 1933).

Table 7 also shows the crossing over from XXY females heterozygous for inversion dl-49. After correcting for the inviable non-disjunctional gametes the values are *v cr* = 4.5, *v bb* = 10.1. Another series of XXY females, of the constitution dl-49 *cm*^{2} *bb*/*y*^{2}, gave (corrected) *y*^{2}*cm*^{2} = 0.5, *y*^{2}*bb* = 13.4, based on 275 regular males and 70 *bb* regular females, respectively. The conclusion seems warranted that the Y somewhat decreases crossing over between the spindle attachment and the inversion.

Inversion yellow-4

According to DUBININ and FRIESEN (1932), the y-4 inversion was found by SEREBROVSKY. It presumably arose as the result of X-ray treatment and is inseparably associated with a mutation of the yellow gene to an allelomorph very closely resembling the original *y*^{1}. As shown below, the leftmost inversion break is located very near to or at the yellow locus (to the left of it according to MULLER and PROKOFJEVA 1935); the rightmost break is located between the genes *fu* and *da*.

Females heterozygous for inversion y-4 give, among their regular offspring, about 2.7 percent of double crossovers within the inversion; about 2.4 percent of the sons of such females are patroclinous. The data from which these percentages are obtained are presented in table 8. Certain of the crossovers appear as singles within the inversion; they are presumably doubles with the second crossover in the short uncontrolled region between *f* and the end of the inversion. The ratio of recovered double crossovers among male offspring of XX mothers (57) to patroclinous males (51) is 1.12:1.

The data from XXY females (table 8) can be compared with those from XX females, since the XXY mothers were sisters of approximately half of the XX mothers and since the two lots of XX data gave similar results (2.8 and 2.3 percent of males patroclinous, 2.7 and 2.0 percent of regular males crossovers). The percentage of crossovers among regular sons of XXY mothers is 6.1, or 5.8 when corrected for exceptional offspring. As in the case of inversion sc-4, the frequency of recovered double crossovers is increased by the presence of a Y chromosome.

TABLE 8

Crossing over in In y-4/sc cv v f and in sister In y-4/sc cv v f/Y females by B or sc B males.

	XX FEMALES		XXY FEMALES	
B ♀♀	2106		548	
+ ♀♀	1		39	
B ♂♂	51		56	
Regular males				
0	1019	1044	287	250
1–2	2	3	3	3
1–3	16	11	9	9
1-(4)	0	1	1	0
2-3	11	8	7	6
2-(4)	3	1	0	0
3-(4)	0	1	0	0
Total regular males		2120		575

Inversion bobbed deficiency

From X-ray treated males SIVERTZEV-DOBZHANSKY and DOBZHANSKY (1933) got an X chromosome carrying a deficiency for the proximal third of the somatic metaphase X chromosome, and a small-wing (*sl*) allelomorph. Females heterozygous for this and a normal X chromosome gave very low crossover values, a result ascribed by the above workers to the presence of the deficiency in heterozygous condition. We have obtained clear evidence that the bobbed deficiency chromosome carries an inverted segment extending from between *rb* and *rg* to between *cr* and the spindle attachment.

SIVERTZEV-DOBZHANSKY and DOBZHANSKY published data on crossing over in females heterozygous for Df (bb). Our data are substantially the same (table 9). The *y-ec* interval lies outside the inversion and shows (in XX females), 0.8 percent of recovered single crossovers. This is a marked reduction as compared with the standard crossover value for this interval. The region from *ec* to *ct* is partly outside and partly within the inversion. To get the frequency of recovered double crossovers within the inversion, apparent singles in this region (four in number) are assumed to be actually

doubles since the greater part of the region must lie inside the inversion. Recovered doubles within the inversion, assuming apparent singles to be doubles with the second single in the unmarked *f*-spindle attachment region, constitute 3.9 percent of the regular males. The relation between double crossovers and patroclinous males will be considered in connection with the mechanism of disjunction in inversions.

TABLE 9

Data from the cross sc sl² Df(bb)/ec ct⁶ g² f×w B. The XX and XXY females were sisters.

	XX FEMALES		XXY FEMALES	
B ♀♀	1244		1622	
+ ♀♀	0		255	
w B ♂♂	21		292	
Regular males				
0	443	601	695	774
1	5	4	4	10
2–(6)	1	3	5	2
3–(6)	12	10	13	17
4–(6)	1	2	4	1
5–(6)	1	0	2	0
2–3	0	0	1	0
2–4	0	0	0	1
2–5	0	1	2	1
3–4	1	6	3	0
3–5	3	1	5	3
4–5	1	0	0	0
1–3–(6)	0	0	0	1
Total regular males		1096		1544

Females heterozygous for Df (bb) and carrying a Y chromosome gave about the same frequency of double crossovers within the inversion as did their XX sisters, 3.7 (corrected for non-disjunction) as compared with 3.9 percent. There is no indication here of an increase in crossing over in the presence of a Y chromosome such as that shown by the sc-4 and y-4 inversions.

Crossover data from XXY females homozygous for Df (bb) are given in table 10. (XX homozygotes do not survive, as shown by SIVERTZEV-DOBZHANSKY and DOBZHANSKY.) The *y-f* interval gives a crossover value of 37.1 percent which is higher than is given by this segment in its normal position in the chromosome (8 plus 10 units). The remaining intervals show less than normal crossing over with the decrease becoming more marked toward the spindle attachment. Presumably we are here dealing with the so-called spindle attachment effect, that is, segments moved away from the spindle attachment show increased crossing over; distal

segments moved near the spindle fiber show a decrease in crossing over (OFFERMAN and MULLER 1932; BEADLE 1932).

TABLE 10

Data from the cross $y^2\ f\ v\ Df(bb)/sc\ g^2\ ct^6\ Df(bb)/Y \times w\ B$.

		B ♀♀	2269		
		+ ♀♀	69		
		w B ♂♂	84		
Regular males					
0	478	434	1–4	15	17
1	304	312	2–3	0	2
2	72	107	2–4	3	5
3	46	52	3–4	1	1
4	33	37	1–2–4	1	1
1–2	23	19	1–2–3	1	0
1–3	25	27			
			Total regular males		2016
		Region	Percentage of crossovers		
		1	37.1		
		2	11.6		
		3	7.7		
		4	5.7		

COMBINATIONS OF DIFFERENT INVERSIONS

In females carrying overlapping inversions, single crossovers within the region common to the two inverted segments should give chromatids with single spindle attachments, in contrast to the chromatids with two or with no spindle attachments resulting from single crossing over within the inverted segment in a female heterozygous for a single inversion. If two overlapping inversions are not too different in length such single crossovers should be viable in the heterozygote. Actually we know this to be the case in several combinations of X chromosome inversions. GERSHENSON (1932) has reported a bobbed deficiency chromosome resulting from single crossing over between In sc-4 and In sc-8.

Crossovers between different inversions will of course give different results depending on the relative positions of the inversion points. Thus, representing the normal sequence of segments of a chromosome such as *A B C D E F*, inversions differing only at one end, $\frac{A\,B\,D\,C\,E\,F}{A\,B\,E\,D\,C\,F}$, will give crossovers *A B D C F*, a single deficiency and *A B E D C E F*, a single net duplication (fig. 2). If both ends differ in position, there are two more possibilities. Thus $\frac{A\,B\,D\,C\,E\,F}{A\,E\,D\,C\,B\,F}$ gives *A B D C B F*, a duplication

for *B* and a deficiency for *E*, and the complementary duplication-deficiency *A E D E F* (fig. 3). The third possibility $\frac{A\,B\,E\,D\,C\,F}{A\,D\,C\,B\,E\,F}$ gives the double duplication *A B B D C B E F* and the double deficiency *A D C F* (fig. 4). We shall consider examples of all of these possibilities.

TABLE 11

Chromosomes resulting from single crossing over within common inverted regions.

SOURCE OF		DUPLICATION	DEFICIENCY	♀	♂
LEFT END	RIGHT END	FOR	FOR	HETEROZYGOUS FOR NORMAL CHROMOSOME	
sc-4	sc-7	*cl-cr*	none	Inviable	Inviable
sc-4	sc-8	*sc*	*bb*	Normal; fertile	Normal; fertile; inviable without Y
sc-4	Clb	*fu-cr*	*slv-ec*	Inviable	Inviable
sc-4	dl-49	*g-cr*	*slv-rb*	Inviable	Inviable
sc-4	y-4	*y-sc, cr*	none	Normal	Normal
sc-7	sc-4	none	*cl-cr*	Inviable	Inviable
sc-7	sc-8	*sc*	*cl-bb*	Inviable	Inviable
sc-8	sc-4	*bb*	*sc*	Fertile; legs often abnormal	Nearly completely lethal; sterile; extreme sc
sc-8	sc-7	*cl-bb*	*sc*	Inviable	Inviable
sc-8	Clb	*fu-bb*	*sc-ec*	Inviable	Inviable
sc-8	dl-49	*g-bb*	*sc-rb*	Inviable	Inviable
sc-8	y-4	*y-ac, cr-bb*	none	*Hw* effect of sc-8; fertile	*Hw* effect of sc-8
Clb	sc-4	*slv-ec*	*fu-cr*	Inviable	Inviable
Clb	sc-8	*sc-ec*	*fu-bb*	Inviable	Inviable
Clb	dl-49	*g-sy*	*bi-rb*	Inviable	Inviable
Clb	y-4	*y-ec*	*fu*	Fertile; abnormal eyes, wings, hairs	Inviable
Clb	Df(bb)	none	*bi-rb, fu-bb*	Poorly viable; sterile; minute bristles	Inviable
dl-49	sc-4	*slv-rb*	*g-cr*	Inviable	Inviable
dl-49	sc-8	*sc-rb*	*g-bb*	Inviable	Inviable
dl-49	Clb	*bi-rb*	*g-sy*	Inviable	Inviable
dl-49	y-4	*y-rb*	*g-fu*	Inviable	Inviable
y-4	sc-4	none	*y-sc, cr*	Poorly viable; fertile; minute bristles	Inviable
y-4	sc-8	none	*y-ac cr-bb*	Poorly viable; fertile; minute bristles	Inviable
y-4	Clb	*fu*	*y-ec*	Inviable	Inviable
y-4	dl-49	*g-fu*	*y-rb*	Inviable	Inviable
y-4	Df(bb)	none	*y-rb cr*	Inviable	Inviable
Df(bb)	Clb	*bi-rb, fu-cr*	none	Fertile; wings slightly narrowed	Sterile; wings notched at tip
Df(bb)	y-4	*y-rb, cr*	none	Fertile; wings narrow, bristles abnormal	Inviable

The recoverable single crossovers with either one or two deficient segments are useful in determining genetically the position of the inversion points. Thus if such a crossover shows a deficiency for gene B but not for genes *A* and *C*, we can say that one inversion end is located between genes *A* and *B*, the other between *B* and *C*. The precision of this method is limited only by the extent and accuracy of the genetic map and by the fact that only recessive mutant effects are available for deficiency tests.

From many combinations of two inversions we have collected data on non-disjunction (table 14) which are discussed below.

Table 11 gives a summary of the available information concerning the properties of the chromosomes derived from single crossing over in the region common to two inversions. Where we have recorded a given crossover chromosome as being inviable, this is to be understood as meaning "under ordinary conditions." It is quite likely that some of the types in question could be brought to maturity by special culture techniques, which we have not used in any case.

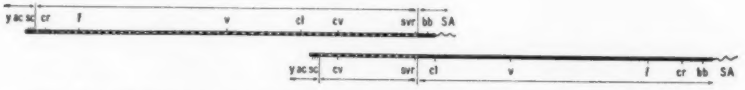

FIGURE 2.—Inv sc-4 (above)/Inv sc-7 (below). Diagram showing conjugation of the common inverted regions. The arrows point in the direction in which the loci are arranged in "normal" chromosomes, reading from the distal end to the spindle attachment.

Inversion sc-4/Inversion sc-7

In this case both left inversion points lie very close to *sc*, and to the right of it; they must be nearly or exactly at the same level. The right point is much further from this level in sc 4. Singles within the common inverted region would be either duplications or deficiencies for the long section from near *cv* to *cr* (fig. 2), and it is accordingly in agreement with expectation that they were not recovered in crosses to normal males. The only other type of crossover that might be expected to appear is the double within this *cv-cr* section, and a few of these were obtained.

From *y sc*^{4} *cv f*/*sc*^{7} *w*^{a} ♀ (×various ♂♂) were obtained 459 regular ♀♀ (no crossovers were observed in those 2 of the 4 cultures where they could have been identified, but detailed counts were not recorded), 228 *sc w*^{a}♂♂, 176 *y sc cv f*♂♂, 8 *sc w*^{a} *f*♂♂, 3 *y sc cv*♂♂,

By mating sc-4/sc-7 heterozygotes to translocation 1,2-7 (break between *rb* and *cv* in X and attached to the right of *sp* in II) or translocation 1, 3–3 (break between *rb* and *cv* in X, attached to the right of *ca* in III) we were able to save the crossovers in the common inverted segment which are deficient for the long segment from the right end of the sc-7 inversion

(near *cv*) to the spindle attachment (fig. 2). From the cross *y sc*^{4} *v f cr*/ *sc*^{7} *w*^{a} *fa*^{2} *sn v* by T 1, 2–7, 455 normal ♀ ♀, 2+♂♂, 181 *y sc*^{4} *v f cr*♂♂, 184 *sc w*^{a} *fa sn v*♂♂ were obtained. In addition there were 16 males carrying the deficiency crossover plus the proximal X segment from the translocation. Of these 13 were *sc*, 1 was *sc w*^{a} *fa* and 2 were *sc fa*. All were strong scutes, otherwise normal. Of these 11 were tested; none was fertile (they are of course expected to be XO). The 16 males constitute 4.05 percent of the regular males, which would indicate, since the contrary class is lost, a frequency of 8.1 percent crossing over in the common inverted segment. It is assumed that the translocation males produce four types of gametes in equal numbers.

Females of the same constitution as above mated to T 1, 3–3 males gave essentially similar results. There were 407 regular non-crossover males and 6 crossovers of the type considered above. Here the frequency of single crossovers, corrected for the class not recovered, is 2.9 percent. Three of these males were tested and, as expected, were sterile.

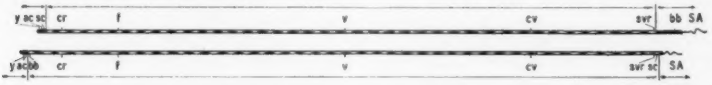

FIGURE 3.—Inv sc-4 (above)/Inv sc-8 (below). Conjugation of the common inverted regions.

In both of the above cases the percentages of recovered crossovers indicate minimum values of crossing over in the common inverted segment. Since the recovered crossover individuals carry a net duplication (difference between sc-7 right break and translocation break) they are probably lower in viability than non-crossover males. Likewise if the translocations give more regular than non-disjunctional gametes the observed frequency of crossing over will be lower than the real value.

Inversion sc-4/Inversion sc-8

The sc-8 inversion is slightly longer than the sc-4 one at both ends, the resulting single crossovers carrying either a deficiency for *sc* and a duplication for *bb* or a duplication for *sc* and a deficiency for *bb*. The conclusions as to their limits depend in part on the results obtained from the heterozygote here under discussion, so the argument may now be presented.

GERSHENSON (1932) has already described the crossover that receives the left end of sc-4 and the right end of sc-8 (fig. 3), showing that it acts as though it carried a lethal allelomorph of *bb*, both the lethal and the bobbed effects being suppressed by a Y. This shows that the *bb* locus is absent, that is, that it is present in the inverted portion of sc-8 and in the uninverted portion of sc-4, both of which are absent in this crossover.

GERSHENSON has also studied the deficient chromosome cytologically in the oögonia of heterozygous females; he finds it to be reduced in length by about one quarter. This can only mean that a large section (not far from half) of the inert region is included in In sc-8 and not in In sc-4. The latter presumably includes little or no inert region. Finally, GERSHENSON showed that this chromosome did not carry a deficiency for *cr*. It follows that In sc-4 has its right break between *cr* and *bb*, while In sc-8 has it to the right of *bb*, conclusions which our own data confirm.

The other crossover, that must carry two *bb* loci, is usually lethal in the male. We have found that it does not carry a deficiency for *ac*, *slv*, or *br*. These three loci must thence be alike in the two inversions, that is, either in both or outside of both, since the crossover studied by GERSHENSON also carries no deficiency for them. When tested against scute allelomorphs this chromosome behaves as an extreme scute, but so does sc-4 itself, so this is not a critical result. However, we have been able to obtain a few males carrying this chromosome. Occasionally they emerge but only live a few hours. Examination of their bristles shows that they have many fewer than sc-4 males; the only named ones observed were the inner verticals, posterior supra-alars, and the dorsocentrals, that is, the "achaete" as opposed to the "scute" bristles, and even these were frequently absent. Dr. J. SCHULTZ informs us that a study of the salivary gland chromosomes also indicates that the sc-8 break is to the left of the *sc* band, the sc-4 one to the right of it. It must be concluded that these males represent the occasional survival of specimens in which the scute locus is wholly absent.

Females heterozygous for this chromosome often have some of their legs abnormal. The abnormality, which is most frequent in the posterior pair, may consist in bifurcation, shortening and twisting, or basal fusion of the two members of one pair.

The above results show that In sc-4 runs from a point between *sc* and *slv* to a point between *cr* and *bb*; In sc-8 from a point between *ac* and *sc* to a point between *bb* and the spindle attachment.

TABLE 12

	f v cv w^a (sc-8) × various ♂♂			
♀	*y sc*^{4}			
Except. ♂♂	456		1, 2	1 3
Regular ♂♂	0		1, 3	7 3
0	52	76	1, 4	1 1
1	0	28	2, 4	2 3
2	1	37	3, 4	3 1
3	0	30	1, 2, 4	0 1
4	0	10	Total ♂♂	260

Table 12 shows the crossing over from sc-4/sc-8. Since one type of single crossover is practically lethal, the simplest way of calculating crossing over seems to be to use in each case only the larger of the two contrary classes. If this is done the values become: total 201; *y-f*, 19.9; *f-v*, 17.9; *v-cv*, 21.4; *cv-w*^{a}, 9.0. These values are, as expected, not different from those for sc-8/sc-8 or sc-8/Df sc-8. Classification for *sc*^{8} was not attempted. The *y* males were typical *sc*^{4}; the *y w*^{a} were nearly wild-type for bristles, carrying both *sc*^{4} and *sc*^{8}.

Both of the crossovers recovered from sc-4/sc-8 have been tested for crossing over and disjunction, and have given the expected results. The one with the left end of In sc-8 (the sc deficiency) when tested against a normal chromosome gave the following results: Df (sc) *f*/*y w*^{a} ♀ × various males: 453 regular ♀ ♀, no exceptions; 337 *y w*^{a} ♂ ♂, 7 *sc* (extreme) *f* ♂ ♂, 17 *y w*^{a} *f* ♂ ♂, 1 extreme *sc* ♂, 1 *y* ♂, 47 exceptional ♂ ♂. (In some cases *sc*^{1}, *sc*^{10}, or *slv* were used instead of *y*.) Calculating the frequency of exceptional males by doubling the *y* class (since the deficiency is nearly lethal), there were 47/767 or 6.1 percent exceptions. In other words this chromosome gives results both as to crossing over and as to disjunction comparable to those shown by the inversions from which it was derived. This result is confirmed by a small series in which the crossover was tested against In sc-8. Here there was about 32 percent crossing over between *sc* and *f*, and only 0.7 percent exceptional males were produced.

The other crossover (*sc* duplication, *bb* deficiency) behaves similarly. Females of the constitution *y w*^{a} *cv* Df(bb)/*f* gave 347 regular sons (of which 10 were crossovers, 3 clearly doubles and the others presumably so) and 11 exceptional sons. The totals for all experiments of this type show 12/391 = 3.1 percent exceptions. This same chromosome was also tested against In sc-4. There resulted 826 regular ♀ ♀, no exceptional ♀, 657 regular ♂ ♂, 4 exceptional ♂ ♂. Among the regular males crossing over could be checked, and the following values were obtained: *sc-w*^{a}, 0.5; *w*^{a}*-cv*, 7.0; *cv-v*, 18.3; *v-f*, 21.3. Here again the crossover, as expected, behaves much like the inversions from which it was derived.

Inversion sc-4/Inversion ClB

The ClB inversion lies wholly within that of sc-4. ClB/+ gives very few crossovers, so that doubles would be expected to be rare here, and none was found. Of the singles within the common inverted region, one type should be a deficiency for *slv-ec* and a duplication for *fu;* the other should be a duplication for *slv-ec*, and a deficiency for *fu*. Neither was obtained; evidently both are inviable in males and also in heterozygous females.

From ClB (*sc v B*)/*y sc*^{4} *cv f* ♀ × *y*^{2} *cv v f* ♂ were obtained 254 *v B* ♀ ♀, 238 *y cv f* ♀ ♀, 2 *sc B* (exceptional) ♀ ♀, 182 *y sc cv f* ♂ ♂, 1 *y*^{2} *cv v*

f (exceptional) ♂. An XXY ♀ of the same constitution gave 27 *v B* ♀ ♀, 33 *y cv f* ♀ ♀, 61 *sc B* ♀ ♀, 24 *y sc cv f* ♂ ♂ and 52 y^2 *cv v f* ♂ ♂.

Inversion sc-4/Inversion dl-49

Scute-4 inversion includes the segment from just to the right of *sc* to between *cr* and *bb*, the greater portion of the chromosome (fig. 1). Delta-49 extends from between *rb* and *cv* to between *fw* and *f* and is consequently entirely included within the sc-4 inversion. From the cross *y* sc-4/dl-49 *y-Hw* cm^2 m^2 g^3 × *l v m* g^2, 1,752 males were recorded, all non-crossovers. This result might have been expected from our knowledge of the behavior of females heterozygous for each of these inversions separately. Doubles within the sc-4 inversion and outside In dl-49 either do not occur or are very rare. Since heterozygous dl-49 gives single crossovers within the inversion (p. 587), it is probable that in the sc-4—dl-49 combination some singles occur in the inverted segment common to the two inversions. Such crossovers result in either a *slv-rb* duplication and *f-cr* deficiency or the complementary deficiency-duplication. Both of these products would be expected to be inviable.

XX females heterozygous for In sc-4 and In dl-49 give few or no exceptional daughters and a frequency of exceptional sons not significantly higher than normal.

Inversion sc-4/Inversion y-4

Inversion sc-4 runs from a point between *sc* and *slv* to a point between *cr* and *bb;* In y-4 from a point near *y* (to the left of *ac*) to a point between *fu* and *cr*. One of the single crossovers is deficient for *y* (?) *ac-sc* and *cr;* the other is a duplication for both these sections. The former is lethal in males but survives occasionally in heterozygous females as a minute-bristled type. The latter (duplication) crossover is viable in offspring of both sexes.

Owing to the presence of *y* in both inverted chromosomes and of an extreme *sc* allelomorph in sc-4, the tests for deficiencies for these two loci are inconclusive. Fully satisfactory tests have shown, however, that the minute crossover is deficient for the loci *ac* and *cr*, not for *rst*, *pn*, *sy*, nor for *od*. The other (duplication) crossover showed no deficiency effects for any of these loci. That is, the inversions differ with respect to *ac* and *cr;* In sc-4 was shown above to include *cr* but not *ac*. Hence y-4 includes *ac* but not *cr*. Both include *rst* and *pn*, as follows from this analysis and from the direct test. In sc-4 includes *sy* and *od;* therefore In y-4 has its right break between these two and *cr;* other data show that it is also to the right of *fu* which is 0.3 units to the right of *od* and *sy*.

The crossing over in In sc-4/In y-4 is similar to that in In sc-4/In sc-8. Here again one single crossover class of males dies, and the data have been treated by using the larger member of each pair of contrary classes. The results are then: total 433; *sc-f*, 20.3; *f-v*, 7.9; *v-cv*, 11.3; *cv-w*^{a}, 10.2; *w*^{a}*-sc*, 1.2.

The *w*^{a} *sc* value appears here and not in the corresponding series for In sc-4/In sc-8 because In y-4 carries a *sc* gene that is clearly dominant to *sc*^{4}, whereas the allelomorph present in In sc-8 gives with *sc*^{4} a variable type not always clearly separable from *sc*^{4}.

Data from XXY In sc-4/In y-4 show the same type of crossing over and roughly the same amount; the experiments however include too few flies to be valuable for detailed comparisons.

Inversion sc-7/Inversion sc-8

This combination of inversions is essentially the same as sc-4/sc-7 already discussed. Females heterozygous for the two inversions give progeny carrying chromosomes derived by crossing over within the common inverted segment. As in the case of the sc-4/sc-7 both of these crossovers are inviable. Doubles within the sc-8 inversion but outside the short sc-7 inversion are recovered.

From the cross sc-8 *w*^{a}/sc-8 *v*/Y by various males the following offspring were recovered:

Regular	♀♀	589	
Exceptional	♀♀	70	
Exceptional	♂♂	103	
Regular	♂♂		
0		247	246
2-(3)		11	10

In addition there was one exceptional male expected to be *y*^{2} *cv v B* which did not show *y* but which did show a hairy wing effect. This male was sterile. Evidently he carried a duplication for *y*.

From crosses of sc-7/sc-8 to T 1, 2–7 or T 1, 3–3 males (as in the case of sc-4/sc-7) males were recovered which carried the crossover deficient for the long segment from the right break in sc-7 to *bb*. These were enabled to survive by the proximal X segment from the translocation males. Such males differ from those obtained from sc-4/sc-7 (fig. 2) in not carrying a *bb* duplication and in carrying a duplication for the *sc* locus. From the cross sc-8 *w*^{a} *cv v f*/sc-7 *w*^{a} *fa*^{2} *sn v* by translocation males the following flies were recorded:

		♂♂ T 1,2–7	♂♂ T 1,3–3
Regular females		844	867
matroclinous	♀♀	1	0
v minute	♀♀	0	1
Patroclinous	♂♂	4	5
Regular	♂♂	796	728
sc w^a	♂♂	17	19
sc w^a *fa*	♂♂	0	1

The *v* minute female recorded above carried the long crossover chromosome, tandem attached-X chromosomes deficient for the common inverted segment of the parent inversions. This deficiency was partly covered by the distal X segment from the translocation.

The *sc* w^a and *sc* w^a *fa* males carry the short crossover chromosome. The actual frequency was 2.1 and 2.7 percent of regular males. These males carry both the sc^7 and sc^8 genes and were intermediate between sc^7 and sc^8 males for the scute character. Their wings were spread. All of them that were tested (seven) were sterile as expected and were presumably XO.

Assuming that the translocation males produce four types of gametes in equal numbers, and that the above mentioned males have normal viability the true percentages of crossovers in the common inverted segment would be obtained by doubling the crossover male classes (the contrary crossover is not recovered) which would give values of 4.1 and 5.2 percent. Since these males are almost certainly of lower viability than the regular males, these percentages represent minimum values. The real value is probably considerably higher.

Inversion sc-7/Inversion ClB

These two inversions overlap in the rather short region from *bi* to near *cv*. Singles would be expected to be rare within this region and would be inviable. None was recovered. From the behavior of each when heterozygous for a normal chromosome it is inferred that few crossovers of any kind would be recovered. In fact, sc-7 w^a/ClB *sc v B* females gave 327 regular sons with one crossover which was sc^7 w^a *v* that is, a double within that part of In ClB not common to In sc-7. Similar XXY females gave 270 regular males with no crossovers.

Inversion sc-7/Inversion dl-49

The right break in In sc-7 is near *cv*, the left break in dl-49 between *rb* and *cv*. In the absence of more accurate information we cannot say definitely whether or not these two inversions overlap although it seems more probable that there is a short overlapping segment. The data from

sc-7 w^a/dl-49 cm^2 XX females by *w B* males are limited; 157 males show no crossovers but the region to the right of the dl-49 inversion where one might expect a low frequency of crossovers is not under control. From XXY mothers, similarly marked, 206 non-crossover males were recorded.

Inversion sc-7/Inversion Df(bb)

In the combination of In sc-7 and In Df(bb) there is probably a short overlapping segment between *rb* and *cv*. From the cross sc-7 $w^a/y\ sl^2$ Df (bb)- × *sc ec ct l* g^2 *sl*, 1651 regular males were recorded of which 19 or 1.15 percent were apparent single crossovers between w^a and *sl*. Presumably all of these were actually doubles with the second crossover in the uncontrolled region between *sl* and *bb*. Several sc-7 w^a sl^2 males and *sc sl* females were tested and found not to carry Df (bb) showing that they were double crossovers. Presumably an appreciable number of undetected double crossovers occurred in the rather long unmarked region between the right end of In sc-7 and the locus of *sl*.

In the above cross 22 patroclinous males were recorded. Exceptional females could not be distinguished from one crossover class but since there were only 18 females in this class and 12 in the contrary crossover class, the number of exceptional females could not have been large.

Inversion sc-8/Inversion ClB

Inversion ClB is wholly within the limits of In sc-8, and few or no recovered crossovers of any kind are to be expected—the case being very similar to that of In sc-4/In ClB. In fact none was obtained among 21 regular sons of XX females or 160 sons from XXY females.

Inversion sc-8/Inversion dl-49

This combination is essentially similar to the combination of In sc-4 and In dl-49 already considered; the discussion given there applies in the present combination. From the cross sc-8 w^a/dl-49 cm^2 by *w B*, 1742 non-crossover regular males were recorded and 6 patroclinous males. From 5 additional cultures not recorded in detail, 2 apparent w^a *cm* males were obtained. These proved, on testing, to be the result of mutation of an eye color gene in the dl-49 chromosome rather than of crossing over. The locus of the mutation was not determined. From XXY females of the above constitution mated to *w B* males 576 non-crossover regular males and 444 patroclinous males were recorded.

STONE AND THOMAS (1935) also studied this combination. They obtained one double crossover (outside of the dl-49 inversion, inside of the sc-8 one) in experiments carried out at 30°C.

Inversion sc-8/Inversion y-4

Inversion y-4 extends to the left further than In sc-8 by the locus of *ac* (and *y*?); In sc-8 extends further to the right by the *cr- bb* section. No crossovers are to be expected outside the inversions; of the singles within the common inverted region, one is a duplication for *ac* (and *y*?) and for *cr-bb;* the other is a deficiency for both these sections. The latter is lethal in males, the former survives; in heterozygous females the former (duplication) is fully viable, the latter gives a minute-bristled individual that has reduced viability.

Tests against recessives show that the "minute" crossover is deficient for *ac* and *cr*, not for *slv;* the other one (duplication) is deficient for none of these loci. Therefore the inversions differ in that one includes *cr*, the other does not; likewise they differ for *ac;* both or neither include *slv*. These results are in agreement with the conclusions from sc-4/sc-8 and sc-4/y-4, which show that *slv* is included in both, *cr* in sc-8 but not in y-4, *ac* in y-4 but not in sc-8.

The crossing over tests for this combination show results similar to those from sc-4/y-4 but they are too scanty to permit detailed comparisons.

The duplication crossover, tested against a normal chromosome gave no exceptions among 314 daughters, 18 among 332 sons (5.4 percent). These values, as expected, are comparable to those from the In sc-4—In sc-8 crossovers.

Inversion ClB/Inversion dl-49

The delta-49 inversion is entirely included within the ClB inversion. From the cross ClB *sc v sl B*/dl-49 *cm*^{2} *bb*^{x}×*sc cv v f cr*, 629 regular non-crossover males (*cm*^{2}) and 2 patroclinous males were obtained. Among 1266 females, 3 (0.24 per cent) were crossovers between *sc* and the left break of ClB; one was of the constitution *sc B* presumably a primary exception equational for the scute gene. XXY females of this combination were not studied.

Single crossovers in the inverted segment common to ClB and dl-49 presumably occur but are evidently inviable.

Inversion ClB/Inversion y-4

The right inversion points here are very similar, differing only in that In y-4 includes *fu*, In ClB does not. At the left In y-4 is considerably longer. Crossing over might occur between the inversions and the spindle attachment, but tests have not been made. Of the singles within the common inverted region, one gives a long deficiency for (*y*?) *ac* to *ec*, and a short duplication for *fu*, the other has the corresponding duplication and

deficiency. Both crossovers are lethal in males. The one with the long (*ac-ec*) deficiency is also lethal in heterozygous females; the other is viable. This latter crossover usually carries the *B* gene of ClB, and in that case the resulting *B*/+ females have very narrow bar eyes similar to those of *B*/*B*. They also have irregularly arranged acrostical hairs, and their wings are slightly reduced in size and are less convex than is normal on the posterior margin. Tests of this crossover chromosome show that it carries a deficiency for *fu*, not for *y*, *ac*, *br*, *w*, *ec*, *f*, *vb*, *sy*, *od*, *cr*. The negative results could all have been predicted from conclusions already established in this paper; the positive case constitutes our proof that *fu* is in the inverted section of y-4, not in that of ClB.

From y-4 w^a/ClB *sc v B* only one of the 280 regular sons was a crossover. This one, y^4 w^a *v*, was a double within the common inverted region. The females from these same mothers (excluding the mating to *fu* ♂ because of the low viability of *fu*/Df) gave 218 broad bar non-crossover ♀ ♀, 237

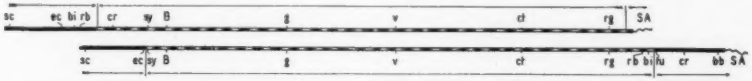

FIGURE 4.—In Df (bb) (above)/In ClB (below). Conjugation of the common inverted regions.

not-bar non-crossover ♀ ♀ and 72 narrow bar crossover ♀ ♀. From the mating to *fu* ♂ one of the 6 *fu*/Df daughters was not-bar, and must have resulted from crossing over between *B* and the inversion point of ClB, a distance of less than two units on the standard map.

Inversion ClB/Inversion Df (bb)

The left break of ClB is between *bi* and *ec*, the right between *sy* and *fu*. The left break of Df (bb) is between *rb* and *rg*, the right between *cr* and *bb*. That these statements are correct will be shown below from studies of single crossovers between ClB and Df (bb). Both breaks of Df (bb) are to the right of those of ClB. Consequently one single crossover should give duplications for the *bi-rb* and *fu-cr* segments (fig. 4). The contrary crossover should give a deficiency for these two segments plus the deficiency for bobbed from the Df (bb) chromosome (fig. 4). Both of these crossovers are viable and can be recovered in heterozygous females. The duplication chromosome is viable in the male. Such males are small with wings having a less convex outer margin than normal and usually with one or more notches at the tips; they are sterile. Dissections by Professor DOBZHANSKY show that the testes are collapsed like those of very old males. Females heterozygous for the double deficiency survive as extreme minutes with wings of a characteristic shape. They have normal ovaries as shown by

dissections made by Professor DOBZHANSKY but according to many tests are sterile. Males carrying this deficiency chromosome are inviable.

Tests of the deficiency-carrying crossover which gets the right end from Df (bb) and the left end from ClB give positive evidence that the *rb* and *cr* loci are absent. From crosses of ClB/Df (bb) to *bi* and *fu* males no deficiency heterozygotes were obtained. We conclude that both of these loci are included in the deficiency crossover. The results from ClB/y-4 establish this for *fu*. The number of flies examined was in each case adequate to have given many deficiency crossover females were they not inviable. Similar tests have shown that the *ec*, *rg*, *f*, *bb*, and *sy* loci are not included in these deficiencies. These results confirm the conclusion already drawn that the right break of ClB is between *sy* and *fu*. The left break must lie between *ec* and *bi*. These conclusions confirm and extend those of PAINTER (1934) derived from studies of salivary chromosomes. In a similar way it is clear that the left break of Df (bb) lies between *rb* and *rg* and that the right one lies to the right of carnation.

Frequency and distribution of single crossovers

Among 2010 regular females from ClB/Df (bb) mothers, 48 or 2.4 per cent carried the deficiency crossover. The contrary crossover could not be classified accurately in the females; if they were of equal frequency, the recovered single crossovers would be 4.8 percent of the total. Among 749 regular males, 26 or 3.5 percent carried the double duplication crossover. The contrary class dies but half the non crossovers likewise die because of the ClB lethal so that this value represents a direct measure of the frequency of singles. It is quite certain that both of the above values are much too low; the crossover-carrying individuals in both cases are of very poor viability and no precautions were taken to prevent overcrowding in the cultures.

It is of interest to determine how the single crossovers in the common inverted segment are distributed. In all experiments *v* was carried by the ClB chromosome. We can then separate crossovers in males into those which occurred between *rg* and *v* and those which occurred between *v* and *sy*. When this is done, the following results are obtained:

Crossover interval	Standard map lengths	Number of crossovers
rg to *v*	22+	111
v to *sy*	27.2	141

The ratios of standard map lengths of these intervals is 1:1.24−; that of singles within these regions 1:1.26. We can conclude that the distribution of single crossovers within the common inverted segment is approximately normal.

Crossing over in heterozygotes for the duplication crossover

Crossing over in females heterozygous for the Df (bb)-ClB crossover has been studied in two experiments. Only one of these is reported here. The other, although involving larger numbers of individuals, is not as well controlled for crossing over in different regions. The data from females carrying the duplication chromosome and a bobbed deficiency chromosome are given in table 13.

TABLE 13

Data from the cross y^2 *f v* dup/*sc* g^2 ct^6 *Df (bb)* ♀ ♀ by *w B* ♂♂. *The females of this experiment were sisters of those used in the experiment summarized in Table 10, homozygous Df (bb) of the same constitution.*

		B ♀♀	1471		
		+ ♀♀	0		
		w B ♂♂	5		
*Regular ♂♂					
0	91	411	1–5	0	6
1	99	14	2–3	0	2
2	60	14	2–4	0	3
3	55	7	3–4	0	1
4	31	6	3–5	0	1
5	7	0	1–3–4	1	0
1–2	0	8	1–2–4	1	0
1–3	3	14			
1–4	0	8	Total		844

* In each case the smaller of the two contrary classes represents the males carrying the duplication.

In comparison with the Df (bb) chromosome, the double duplication crossover chromosome carries one net duplication, namely a segment including *rb-bi-f-cr* from the ClB chromosome. This segment together with the bb segment is simply added to the Df (bb) chromosome which of course carries the *bi-rb-cv-f* segment (no difference in arrangement) at the left end. The data of table 13 show the following crossover values as measured in the classes not carrying the duplication:

y-f	19.4
f-g	10.6
g-v	10.5
v-ct	6.4
ct-dup	2.1

For these same regions in homozygous Df (bb) sister females with a Y chromosome the values for the first four regions above (Table 13) are 37.1, 11.6, 7.7, 5.7 respectively. The fifth region cannot be measured in Df (bb) /Df (bb); its standard map length is 10 units.

It is clear from the above that the duplication crossover chromosome crosses over freely with Df(bb), one of the inversions from which it was derived. As compared with Df(bb)/Df(bb)/Y, crossing over is reduced in the *y-f* interval but is the same in the other intervals which can be compared. The reduction in the *y-f* interval is presumably the result of the duplication which is of course homologous with a segment included in the *y-f* interval.

Inversion dl-49/Inversion y-4

From the cross y-4 w^a/dl-49 *y Hw* m^2 g^3 ♀ ♀ by *t v m g* ♂ ♂, 1506 regular sons showed no crossovers. There were two patroclinous males. There were 1,651 regular females, all non-crossovers, and five exceptional females, four from 1 of the nine cultures.

From a cross of XXY females of the above constitution with *w* or *w B* males, 428 regular non-crossover, 487 patroclinous males, 446 regular females and 506 exceptional females were recorded.

It is clear that recoverable crossovers are practically absent in females of this combination, unless they occur between the right break of y-4 inversion and the spindle attachment. Data from attached-X females heterozygous for y-4 (p. 564) indicate that singles in this interval are very rare.

Single crossovers in the segment common to the two inversions, presumably occur and are lethal both in heterozygous females and in males.

Inversion y-4/Inversion Df (bb)

The location of the breaks in both y-4 and Df(bb) have already been discussed; both breaks in Df (bb) are to the right of those of y-4. Consequently single crossovers in the common inverted segment of these two inversions will give either a double duplication or a double deficiency. The latter is inviable both in males and in heterozygous females; the former is viable in females heterozygous for a normal chromosome. Such a female usually has stubby outer verticals, disarranged scutellars, and outer wing margins less convex than normal. If such a duplication female is heterozygous for *B*, the eyes are usually as narrow as those of a female homozygous for *B*. These crossover females are fertile but produce very few offspring. Their viability is good considering the number of loci carried in the two duplications.

From the cross y-4 w^a *cv v* s^2/*sc* sl^2 Df(bb) by *sc B* males there were recorded one exceptional female (+), 330 regular ♀ ♀ (*B* and *sc B*), 4 *sc B* ♂ ♂, 123 *sc sl* ♂ ♂, 85 *y* w^a *cv v s* ♂ ♂ and 38 duplication ♀ ♀. The frequency of crossover females in percent of regular females is 10.7. From sister females of those used in the cross above, but XXY in constitution, mated to *sc B* males, the following offspring were obtained: 264 + ♀ ♀, 390 *B* ♀ ♀, 236 *sc B* ♀ ♀, 272 *sc B* ♂ ♂, 159 *sc sl* ♂ ♂, 198 *y* w^a *sv v s* ♂ ♂,

and 144 duplication ♂♂. Here duplication females constitute 18.7 percent of the regular females. Since only one crossover is recovered here the true percentage of singles from these data will be 18.7 for XX and 19.8 (corrected) for XXY. The frequencies are not significantly different.

Since females heterozygous for the duplication crossover produce very few offspring, few studies of them were made. It is known that crossing over between the duplication chromosome and a normal chromosome is very low and that a few patroclinous males are produced. These results are expected since the crossover chromosome is in effect an inversion plus an intercalated duplication.

NON-DISJUNCTION

Table 14 is a summary of the available data on the production of matroclinous females and patroclinous males.

In many of these experiments the exceptional females could not be distinguished and only the males are recorded, in others the male exceptions were known to have very low viability and only the females are recorded. It follows that the numbers of individuals in the two sexes from a given combination are often not comparable. ClB and Df(sc-8) are lethal in the males; accordingly in all series involving these the observed number of regular males has been doubled in calculating the recorded total, a point to be remembered in judging the significance of the values given. In many of the combinations of two inversions, single crossovers between the two inversions occur; some of these are lethal and others have reduced viability. No corrections have been made for this; therefore in several of these cases if is certain that the totals are too low and the percentages of exceptions too high.

In the case of ClB/+ we have added our own data to those recorded by GERSHENSON (1935) though in the XXY experiments we obtained somewhat higher values than he records. We have excluded the males from his XX experiment in which the father was bb^1, since the exceptions (having no Y) would be inviable. We have also excluded one unexplained XX culture of our own that gave 9 exceptional females to 223 regulars and 11 exceptional males to 133 regulars. We have observed in some other combinations a suspiciously high frequency of cultures that gave more than one exception when others of the same constitution gave none. In no case were the resulting frequencies high enough to be interpreted as due to the presence of an unsuspected Y. The frequencies are about those that result from the presence of a short duplication carrying the X spindle attachment, but we have not studied the descendants of such females with this possibility in mind.

The results of STONE and THOMAS (1935) for sc-8/+ and dl-49/+ have not been included in the table.

TABLE 14

Summary of non-disjunction data.

	XX MOTHERS						XXY MOTHERS					
	FEMALES			MALES			FEMALES			MALES		
	TOTAL	EXC.	% EXC.	TOTAL	EXC.	% EXC.	TOTAL	EXC.	% EXC	TOTAL	EXC.	% EXC.
sc-4/+	6287	1	0.02	5861	337	5.75	933	40	4.3	817	109	13.4
sc-4/sc-4	953	0	0.00	600	0	0.00						
sc-7/+	5386	5	0.09	4919	14	0.28	1518	195	12.9	1697	256	15.1
sc-7/sc-7	1610	0	0.00	1370	1	0.07						
sc-8/+	4703	1	0.02	5138	164	3.20	1310	130	9.9	1847	255	13.8
sc-8/sc-8	574	0	0.00	481	0	0.00	310	4	1.3	252	5	2.0
Df (sc^8)/+	641	2	0.31	1053	47	4.46	85	9	10.6	80	12	15.0
sc-8/Df (sc^8)	631	0	0.00	504	9	0.00						
ClB/+	5693	14	0.25	3438	16	0.47	7478	2729	36.6	7172	2712	37.8
dl-49/+	3238	0	0.00	3168	6	0.19	4355	1985	45.6	4145	1747	42.2
dl-49/dl-49							126	5	4.0	99	12	2.0
y-4/+	2007	1	0.05	2171	51	2.34	587	39	6.8	631	56	8.9
y-4/y-4	206	1	0.49	169	0	0.00						
Df (bb)/+	1244	0	0.00	3437	67	1.95	1877	255	13.6	1836	292	15.9
Df (bb)/Df (bb)							2338	69	2.9	2100	84	4.0
sc-4/sc-7	459	0	0.00	422	7	1.66	341	34	10.0	280	43	15.3
sc-4/sc-8	456	0	0.00	264	0	0.00						
sc-4/dl-49	2084	0	0.00	1755	3	0.17						
sc-4/y-4	1187	3	0.26	954	0	0.00	413	33	8.0	224	30	13.4
sc-7/sc-7	439	0	0.00	350	12	3.43	659	70	10.6	617	103	16.7
sc-7/Df (sc^8)	523	0	0.00	531	7	1.32						
sc-7/ClB	754	0	0.00	658	4	0.61	894	322	35.8	824	284	34.5
sc-7/dl-49	169	2	1.18	157	0	0.00	355	138	38.8	346	140	40.5
sc-7/y-4	479	0	0.00	297	1	0.34						
sc-7/Df (bb)				1673	22	1.31						
sc-8/dl-49	1959	5	0.26	1748	6	0.34	1146	541	47.3	1020	444	43.5
sc-8/y-4	316	0	0.00	240	1	0.42	906	70	7.7	968	134	13.8
ClB/dl-49	1270	4	0.32	1260	2	0.16						
ClB/y-4				561	1	0.18	101	4	4.0	57	5	8.8
ClB/Df (bb)	1557	2	0.13	1200	6	0.50	1622	520	32.0	1351	561	41.5
dl-49/y-4	1656	5	0.32	1508	2	0.13	952	506	53.2	915	487	53.2
y-4/Df (bb)	370	1	0.27	194	4	2.06	1034	264	25.5	629	272	43.2

PRESENCE OF SINGLE CROSSOVERS

Representing a normal X chromosome schematically as *B C D E* a and an homologous chromosome with the *CD* segment inverted as *B D C E* a (a in both cases representing the spindle attachment), then single crossovers in the *CD* segment will give the products (1) *B C D B* (duplication for *B*, deficiency for *E*, and having no spindle attachment) and (2) a *E C D E* a (deficiency for *B*, duplication for *E*, and having two spindle attach-

ments). Product (1) would be expected to be lost because of its lack of a spindle attachment. Product (2), because of its two spindle attachments, should form a tie between the two poles of the first meiotic division. It is known from the cytological studies of McCLINTOCK (1931, 1933) on Zea, MATHER (1934) on Vicia, STONE (1933) on Tulipa, and SMITH (1935) on Trillium that, for these plants, single crossovers do occur between segments relatively inverted and that the results are as described above. In order to understand the mechanism of disjunction in inversion heterozygotes in Drosophila it is essential to know whether such crossovers occur in this organism and, if so, with what frequency. The most direct method of answering these questions, namely, cytological examination as used in the cases cited above, is very difficult in the case of oögenesis in *Drosophila melanogaster*. We have resorted to less direct genetic methods.

From the data already presented on single crossing over in combinations of two overlapping inversions it seems highly probable that single crossovers occur within the inverted segment in females heterozygous for a single inversion. Inversion scute-8 represents an inversion of the entire X chromosome with the exception of the *y* and *ac* loci and the spindle attachment. It can be considered as representing essentially a transfer of the spindle attachment from the right to the left end of the chromosome.

As regards crossing over it should behave essentially like a normal chromosome. In the heterozygote of sc-8 and sc-4 crossing over is practically normal as has already been shown. We can therefore argue that in the heterozygote sc-4/+, single crossovers should be of approximately normal frequency. Similarly in the combination sc-7/sc-8 we have shown that single crossing over occurs in the common inverted segment. From this we can conclude that single crossing over occurs in the inverted segment of sc-7/+. Here the data from the combination sc-7/sc-8 and also from sc-4/sc-7 suggest that the frequency is reduced below that for the sc-7 inverted segment normally arranged. The same general kind of an argument can be made for several of the other combinations considered in the previous section.

In a female heterozygous for a single X chromosome inversion, crossing over can be more or less directly measured by using attached-X females, (see also SIDOROFF, SOKOLOV, and TROFIMOV 1935). By selecting the appropriate crossover from a triploid of the constitution y-4/$\widehat{XX}$ we obtained an attached-X female heterozygous for the y-4 inversion. In such a female, exchange in the inverted segment will give either (1) a closed chromosome carrying a duplication for *cr-bb* and a deficiency for the small segment to the left of *y*, or (2) a chromatid with two spindle attachments plus a chromatid with none (fig. 5). The Y chromosome that is usually

present disjoins from the attached-X chromosomes at the first division. The results described above and shown in figure 5 take place during the second division. The types and relative frequencies of gametes expected

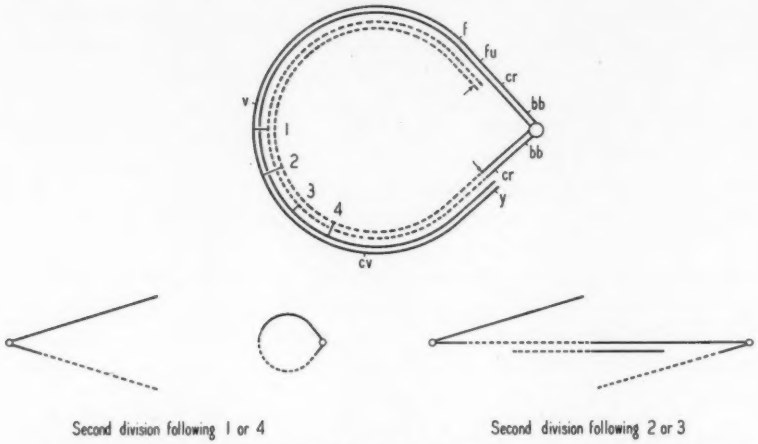

FIGURE 5.—Attached-X, heterozygous for In y-4. Above, conjugation of inverted section. Below, chromatids resulting from the indicated single exchanges. Chromatid with no spindle attachment omitted in lower left.

following single and double exchange are summarized in table 15. Data from the cross y^4cr/y^2v for attached-X females mated to $t\ v\ f$ males are given in table 16.

TABLE 15

EXCHANGE	$\widehat{XX}$ GAMETES				X^c GAMETES	SECOND DIVISIONS WITH CHROMATID TIE	Y GAMETES
	NON-CROSSOVER	RECIPROCAL	EQUATIONAL-a	EQUATIONAL-b			
None	1						1
Single	1				1	2	4
Double* 2-s	1	1	1	1			4
3-s a					2	2	4
3-s b			1	1	2		4
4-s						4	4
Total Doubles	1	1	2	2	4	6	16

* Double exchanges are designated by the number of strands that undergo crossing over. Thus, in a 2-strand (2-s) double exchange, the same two strands cross over at the two levels.

The daughters showing *y* and *cr* carry attached-X chromosomes. Those which show neither *y* nor *cr* carry the closed chromosome derived by single crossing over in the inversion. From the fact that the sex ratio approaches 2 males to 1 female we infer that those cases in which a double spindle attachment arises by crossing over within the inversion result in lethal eggs.

TABLE 16

Progeny of the cross $y^4cr/y^2v\ f\ cr$ attached-X females by $t\ v\ f$ males. Four egg-laying periods; 3, 2, 2 and 3 days.

$\widehat{XX}$ FEMALES		+/X^c FEMALES		MALES	
y^2cr	304	f	137	$t\,v\,f$	1098
$y^2v\ cr$	11	$v\,f$	197	$y\ cr$	1
$y^2v\ f\ cr$	1	v	3		
Total	316		337		

In such cases the X chromosomes are not simply eliminated for we should then expect a ratio of 2+ males to 1 female. Males carrying the closed chromosome are almost completely inviable. The *y cr* male recorded in table 16 apparently carried such a chromosome. This male had narrow wings, other characteristics of duplication-carrying males, was *y* (not y^2) and was sterile. We have not made a cytological study of this closed-X; this has been done by SIDOROV, SOKOLOV, and TROFIMOV (1935) for the closed-X they obtained in the same way (using an unspecified inversion). They have published a drawing of one metaphase plate showing the closed-X.

From the data given in table 16 and the information summarized in table 15, we can make an approximate calculation of the frequencies of single and double exchanges within the inverted segment. Doubles are measured by equationals for genes within the inverted segment (*v* and *f*). Since equationals for one chromosome only are detected, there is one chance in eight of detecting a crossover from a double exchange tetrad. Thus the twelve equationals indicate that there were actually 96 double exchange tetrads. Since a part of the females are eliminated, a corrected total must be used and the most direct way of getting this is to double the number of males. This gives 2196 as a corrected total. The frequency of double exchanges is then 4.4 percent. Using the information in table 15 we can subtract from the observed numbers of individuals those which carry products of double exchange. The remainder should give a measure of single exchanges. Single crossovers are directly recovered as closed chromosomes. The frequency of single exchanges is also measured by the deficiency of females as compared with males. The average of these two

measures of single exchange is 90.8 percent (assuming 50 percent as the value given by recovered closed chromosomes). The summary of the above operations is as follows:

	$\widehat{XX}$ females	X/X^c females	males	Excess of males over females
Observed	316	337	1098	445
Double exchange (4.4%)	36	24		36
Remainder	280	313		409
Single exchange (90.8%)	298.7	248.4		454.9

The two measures of single exchange agree with one another only approximately. However it is quite clear that the frequency of single exchange is high and approaches the frequency characteristic of the segment normally arranged. The distribution of the single crossovers in the inversion heterozygote is indicated by the data, and for the regions *y-v* and *v-f*, is approximately the same as that for normal chromosomes.

Crossing over in the segment between the right break of the inversion and the spindle attachment is very low as indicated by the low frequency of forked equationals. The one y^2 *v f c r* female recorded in table 16 is assumed to have resulted from double exchange within the inverted segment rather than from single exchange to the right of it.

In the case of $\widehat{XX}$ females heterozygous for short inversions, the closed chromosomes resulting from crossing over within the inverted segment are inviable in heterozygous condition and so cannot be recovered unless the duplications and deficiencies are compensated for in some way. However it is clear from the above discussion of attached-X In y-4 heterozygotes that the distortion of the sex ratio in itself can be used as a measure of crossing over.

Early experiments with $\widehat{XX}$ females heterozygous for In sc-7 consistently gave an excess of males over females. The results of three such experiments are summarized in table 17. These experiments indicated relatively high frequencies of exchange in the inverted segment and approximately normal crossing over between the inversion and the spindle-attachment, the latter result being in substantial agreement with those from free-X In sc-7 heterozygotes (p. 557). Since no particular precautions were taken to insure that male and female offspring were of comparable viability in these experiments, stocks more satisfactory with respect to the mutant genes used were made up and the experiments repeated. To decrease viability differences, relatively short egg-laying periods were used.

The results are summarized in tables 18 and 19. These data are in agreement with those from the first experiments in showing that crossing over to the right of the inversion is about normal (the sc-7 chromosome of the females whose progeny are summarized in table 19 apparently carried a semi-lethal mutant of unknown origin and the raw data must be corrected accordingly). However these experiments indicate a lower exchange frequency within the inverted segment than did the earlier ones. The inversion involves a segment 13 to 20 map units long. The data of tables 18 and 19 give crossover values of 9 and 10 percent (one half exchange frequencies).

TABLE 17

Progeny of $\widehat{XX}$ females heterozygous for inversion sc-7 mated to various males.

CONSTITUTION OF DAUGHTERS*	CONSTITUTION OF MOTHERS		
	sc-7 w^a v *f* / y^2 w^a *ec f*	sc-7 w^a *f* / y^2 w^a *ec f*	sc-7 w^a v *B* / y^2 w^a *ec f*
+	267	206	155 (*B*/+)
sc-7	6	45	10 (*B*/+)
y^2 *ec*	53	24	18 (*B*/+)
sc-7 v	53		14 (*B*/+)
y^2 *ec f*			4
sc-7 v, *B*/*B*			5
B/*B*			2
f			1
y^2 *ec*, *B*/*B*			1
Total females	379	275	210
Corrected total females†	385	296	216
Total males	493	483	309
Exchange (%)	29.2	51.6	40.1

* Constitutions are given only with respect to genes heterozygous in the mother.

† Corrected total females obtained by adding to non-crossover phenotype, twice the number of equationals for the sc-7. The correction is for the indicated lower viability of equationals for y^2.

Experiments were made with In dl-49 using the same technique as for the later experiments with In sc-7. The results of two experiments with controls are summarized in table 20. The extent of the distortion of the sex ratio in the two series is not the same. The control experiments indicate that the difference is due to the difference in relative vaibility of the two kinds of males used. Making appropriate corrections of the number of males, the two series indicate exchange values for the inverted segment of 11.5 and 12.5 percent. Exchange in the segment between the right inversion break and the spindle attachment is measured by equationals for

genes within the inversion limits. The values indicated are 3.8 and 10.4 percent for the two series. The cause of the rather large difference is not known. Exchange in the segment to the left of the inversion is measured by equationals for *y* and by equationals for the genes *v* which are not equational for *y*. About all that can be said about the exchange frequency for this terminal segment is that it is low (less than 2 percent).

TABLE 18

Progeny of the cross sc-7 w^acr/y^2cl v f cr attached-X females by wild type males. Egg-laying periods; 3, 2 and 2 days.

FEMALES								TOTAL*	MALES	PERCENT EXCHANGE
cr	sc-7 w^acr	*y cl cr*	*y cl v cr*	*y cl v f cr*	*f cr*	*v f cr*	*v cr*			
629	124	27	79	45	2	3	1	937	1088	18.5

* Total corrected for indicated lower viability of sc-7 w^a

TABLE 19

Progeny of the cross sc-7w^a/y^2v f cr attached-X females by wild type males. Egg-laying periods 3, 2 and 2 days.

FEMALES										TOTAL*	MALES	PERCENT EXCHANGE
+	sc-7w^a	*y*	*y v*	*y v f*	*y v f cr*	*v f*	*f cr*	*f*	*v*			
393	2	14	66	8	1	1	1	1	1	575	679	20.4

* Total corrected as in table 18.

From the evidence considered above it is clear that single crossovers do occur between segments of chromosomes inverted relative to one another. The frequency of such crossovers evidently depends on the length of the

TABLE 20

Progeny of the cross dl-49 cm^2/y^2 v f cr attached-X females×Bar and + males and from control crosses of y^2 v f cr/y^2 v f cr attached-X females by Bar and + males. In each case, three egg-laying periods; 3, 2 and 2 days.

PROGENY	INVERSION		CONTROL	
	×B	×+	×B	×+
+ ♀♀	984	1672		
cm ♀♀	11	46		
y ♀♀	0	1		
y v ♀♀	2	10		
y v f ♀♀	4	28		
y v f cr ♀♀	5	18		
v f cr ♀♀	0	1		
Total ♀♀	1006	1776	590	841
♂♂	1004 (1101)*	2076 (1960)*	538	891

* Numbers in parentheses are corrected totals obtained by multiplying the observed numbers of males by the ratio of females to males in the appropriate control.

inverted segment and its position in the chromosome. These relations will be discussed in more detail in another connection (page 596). In any case we can say that long inversions such as In sc-8, In sc-4, and In y-4 show, with a normal chromosome, a high frequency of single crossing over between the inversion segments. These frequencies are of the same order of magnitude as those characteristic of these same segments arranged in the normal way.

EGG AND LARVAL-PUPAL MORTALITY

We have shown in the preceding section that single crossovers occur with a relatively high frequency in inversion heterozygotes. The question that we shall consider now is whether or not such crossovers result in inviable zygotes. This question can of course be directly answered by determining the amount of mortality in the progeny of heterozygotes for inversions known to give a high frequency of single crossing over within the inverted segment.

Technique

The method that we have used in determining the amount of mortality is essentially the same as that commonly used by other workers (e.g., L. V. MORGAN 1933). Certain modifications were found useful. Paper spoons have usually been used as containers for the medium on which the eggs are collected. They have two disadvantages: (1) the surface of the medium is usually not flat and (2) the depth of the medium varies which often results in drying out around the edges. To overcome these disadvantages small metal (nickel has been found satisfactory) containers about 38 mm long, 17 mm wide and 3 mm deep were made. A handle of the same material about 10 mm wide was soldered to the bottom so that it projected about 3 cm.

The standard cornmeal-molasses-agar medium with the addition of animal charcoal (to increase the contrast between eggs and medium) was liquified, pipetted into the containers and allowed to cool. The flat surface was then painted with a rather heavy yeast suspension. The addition of fermented banana, alcohol or wine was found to be of no advantage. A single female was allowed to deposit eggs on the medium for a period of 24 hours. The container was then removed from the vial and replaced with one containing fresh medium. After removing the container the food was removed from it with a strip of cardboard of appropriate size. The eggs, including those already hatched, were then counted and recorded. The food was placed in a vial at 25°C in a moist incubator for 28 hours after which time the unhatched eggs were counted. The food block was then placed in a standard half pint culture bottle and the flies allowed to develop to maturity. Unless care is taken to have the outside of the food con-

tainer dry there is danger in error from eggs deposited on the sides of the container. The larvae from these eggs may hatch and crawl onto the food block. There is also some error in losing or killing a few larvae in handling the food blocks. The magnitude of these errors can be kept reasonably low with careful manipulation.

Our experience indicates that the percentage of egg mortality is dependent upon the genetic constitution of the mother as well as upon the genetic constitution of the eggs themselves. Thus females from inbred stocks or females homozygous for several recessive genes generally give relatively high mortality regardless of the type of males to which they are mated. Because of this fact, strictly comparable controls cannot be had. To minimize this "residual" egg mortality, crosses between more or less unrelated stocks were made wherever possible and the F_1 females from not overcrowded cultures were used in the egg-laying experiments. Normal controls more or less comparable in genetic constitution were run simultaneously with the experiments on inversion heterozygotes.

Results

The results of our experiments on In sc-4, In sc-8, In y-4, and In dl-49 heterozygotes are summarized in table 21. The answer to the question that we set out to study is quite clear: single crossovers do not give rise to inviable zygotes. In the cases of In sc-4, In sc-8 and In y-4 exchange is approximately normal in frequency. If the distribution of the four strands of a tetrad were random at meiosis, we should expect about half the products to be single crossovers or their equivalent. Since such crossovers are not recovered in the viable zygotes they would have to be eliminated as inviable zygotes. However, it is evident that inviable zygotes do not approach 50 per cent in frequency. Since these inversion heterozygotes produce an appreciable number of patroclinous males (about 3 to 5 percent of the viable zygotes) we know that there should be a corresponding frequency of inviable zygotes (no-X eggs fertilized by Y-carrying sperms). In these cases crossing over could be followed sufficiently well to know that the inversion heterozygotes were giving the usual results. In addition the frequency of patroclinous males was determined and found to be approximately "normal" for the inversion heterozygotes under consideration. When the data are considered in connection with the frequencies of patroclinous males produced (page 595) and with the controls, and when allowance is made for the difference in genetic constitution between inversion heterozygotes and controls, we can conclude that the only zygotes whose death is the direct result of the presence of the inversion in the parent females are those corresponding to patroclinous males and differentiated from them by the sperm.

STONE and THOMAS (1935) have also published egg counts for In sc-8/+ and In dl-49/+. The mortality indicated is higher than in our data, as it is also for their controls. They have also not distinguished between egg mortality and larval-pupal mortality. It seems clear that the lowest adequately established mortality is the one that gives the best picture of the case; and it is to be noted also that STONE and THOMAS conclude, as do we, that single crossovers are not responsible for any detectable portion of the observed mortality.

TABLE 21

Egg and larval-pupal mortality data for inversion heterozygotes.

MATING	TOTAL EGGS	INVIABLE	PERCENT INVIABLE	HATCHED EGGS	ADULT FLIES	PERCENT EMERGENCE OF HATCHED EGGS
y sc-4 *v f cr*/+×*B*	2839	214	7.5	2625	2187	83.4
sc-8 w^a *cv v f*/y^2–×*B*	4190	284	6.8	3906	3745	96.0
y^{2S}/*B* (control for above)×*B*	3947	52	1.3	3895	3741	96.2
sc-8 *B*/w^a *v*/*Y*×y^{2S}	1313	184	14.0	1129	990	87.6
+/w^a *v* (control)×y^{2S}	1018	26	2.5	992	991	99.9
dl-49/+×*B*	1645	51	3.1	1594	1525	95.7
y-4/*v* g^3×*w*	884	68	7.7	816	753	92.4
y-4/y-4×*w*	570	113	19.8	457	375	82.2

Data from XXY In sc-8 heterozygotes are included in table 21. Here both egg and larval-pupal mortality is relatively high. This is of course expected from the fact that here secondary non-disjunction occurs with an appreciable frequency (page 582). The frequency of inviable eggs is of the same order of magnitude as that of exceptional males (YY or YX zygotes, depending upon the sperm) and larval-pupal mortality is of the same order of magnitude as exceptional females (XXX or XXY zygotes, depending upon the sperm).

In connection with studies of sister-strand crossing over, SCHWEITZER and KALISS (1935) have made extensive determinations of egg mortality in inversion heterozygotes. Their results are in agreement with the conclusion we have drawn that single exchanges between inverted segments do not result in inviable zygotes.

THE MECHANISM OF DISJUNCTION IN INVERSION HETEROZYGOTES

It has been shown in preceding sections that single exchanges occur within the inverted segment of inversion heterozygotes, and further, that the crossover products of single exchange are not recovered and do not result in inviable zygotes. It is evident that we must assume that such single crossover chromatids are selectively eliminated during the meiotic process.

It has also been shown that X chromosome inversion heterozygotes give rise to patroclinous males among their progeny. We have implied that the frequency of such exceptional males is a function of double exchange.

The problem that we shall consider is how these two results, (1) elimination of single crossover chromatids and (2) the production of no-X eggs, are brought about. We know from cytological studies on plants (Zea, McCLINTOCK 1933; Tulipa, STONE 1933; Vicia, MATHER 1934; Trillium, SMITH 1935) that double spindle attachment chromatids resulting from crossing over between segments inverted with respect to one another produce chromatin ties between the two poles of the first meiotic division (or under certain conditions to be considered below, between poles of second meiotic spindles). Knowing that the four nuclei resulting from meiosis in

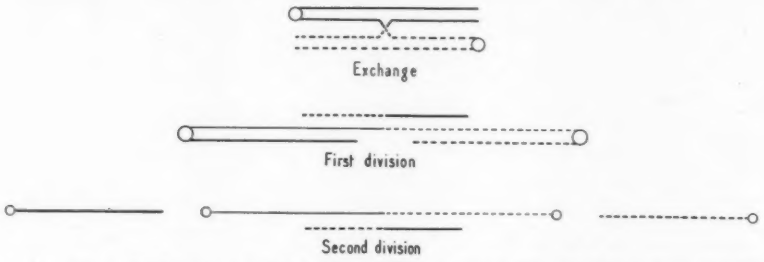

FIGURE 6.—Single exchange within a heterozygous inversion. The upper figure represents the two X's of a female in which one chromosome is practically wholly inverted. At the first meiotic division there results a chromatid tie; this leads to an orientation of the second division such that the two terminal nuclei receive only non-crossover chromatids; one of these is the egg nucleus. The result is the total loss of all single crossover chromatids to the polar body nuclei.

the Drosophila egg lie approximately on a single straight line (HUETTNER 1924), we are prompted to propose the following scheme for the X chromosome of Drosophila.

1. A single chromatid tie at the first meiotic division results in orientation of the spindle attachments in such a manner that only chromatids with a single spindle attachment get into the terminal nuclei, one of which will become the egg nucleus (HUETTNER 1924).

2. A double chromatid tie results in the formation of end nuclei with no X chromosome, and a no-X egg will result.

The behavior of various types of crossover tetrads expected according to this scheme is shown diagrammatically in figures 6 and 7.

As to the precise nature of the orientation of single exchange tetrads or their equivalent we have insufficient information; we know only the end result. It seems reasonable to suppose that the orienting influence of a double attachment chromatid is mechanical. However, we do not know

whether or not such chromatids in Drosophila break during the division as they are known to do in the plants mentioned above. If they break, the orientation probably results from the retardation prior to breakage. Single crossover chromatids without spindle attachments are probably not included in either daughter nucleus but lost in the cytoplasm during division as is known to be the case in plants (McCLINTOCK 1933).

From the diagrammatic representation of the suggested scheme (figs. 6 and 7), it is evident that certain quantitative relations should hold. These

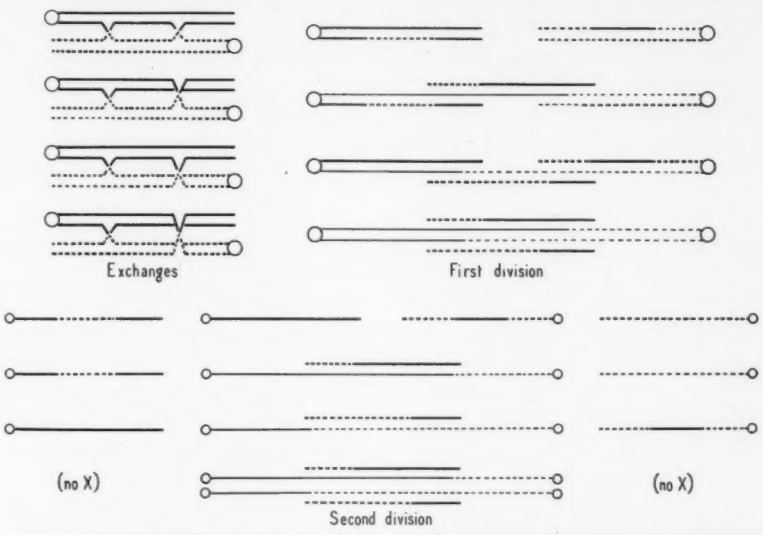

FIGURE 7.—The four possible types of double exchange within a heterozygous inversion. The two-strand exchange (upper row) leads to equal numbers of non-crossover and double crossover chromatids in the terminal nuclei, each of which will be the egg nucleus in half the cases. Three-strand doubles (second and third rows) result in chromatid ties at the first division, and also lead to equal numbers of non-crossover and double crossover chromatids in the egg nuclei. Four-strand double exchange (fourth row) leads to a double tie at the first division, and to no-X egg nuclei.

should serve as tests of the assumptions we have made. The types and frequencies of gametes expected to result from non-, single, double, and triple exchange tetrads are summarized in table 22. It is seen that from double exchange tetrads, double crossover and no-X gametes are expected to occur in the ratio of 3:2. This 3:2 ratio was also found experimentally by STONE and THOMAS (1935) for In sc-8 and for another long inversion that we have not studied. For the longer inversions some triple exchanges presumably occur and these give double crossover and no-X gametes in the ratio of 21:4. Since we have no way of measuring the relative frequencies

of double and triple exchanges in inversion heterozygotes (because triple crossover chromatids must be eliminated) we cannot predict precisely what the ratio of double crossover to no-X eggs should be. However, if we make the assumption that the frequency of triple relative to double exchanges in inversion heterozygotes does not exceed that of normal X chromosomes (about 1:10), then the ratio of double crossover to no-X gametes should lie between 3:2 and 3.4:2.

The numerical data showing the relation of double crossovers to patroclinous males are given in table 23. Half the no-X eggs are lost (fertilization with Y sperm) and in the cases recorded in the table, half the double crossovers were not detected since only male offspring were used to measure crossing over. Hence the zygotic ratios expected are the same as the gametic ratios mentioned above and given in table 22. The observed relative frequencies approach rather closely the ratio 3:2. In no case is the deviation from 3:2 statistically significant. The totals approach very closely a

TABLE 22

Relative frequencies of types of gametes produced following single, double, and triple exchange within the inverted segment of an X chromosome tetrad.

EXCHANGE	DESIGNATION*	NON-CROSSOVER	DOUBLE CROSSOVER	NO-X EGG
None		1	0	0
Single		1	0	0
Double	2 (1)	1	1	
	3 (2)	2	2	
	4 (1)			2
	Total	3	3	2
Triple	2, 3, 3 (4)	4	4	
	2, 4, 4 (2)		4	
	2, 2, 2 (1)	2		
	3, 4, 3 (2)			4
	3, 2, 3 (2)	1	3	
	3, 3, 4 (4)		8	
	4, 2, 4 (1)		2	
	Total	7	21	4

* Doubles are designated by the number of strands involved in the two exchanges; triples in the same way by considering them as three doubles, taking successive exchanges a, b and c in combinations of two in the order a-b, a-c, and b-c, and in this case, disregarding the direction (a-b-c is equivalent to c-b-a). Relative frequencies of different types among doubles or among triples are indicated by numbers in parentheses.

The frequencies of gametes (totals of last three columns) must in each case be proportional to the frequencies of occurrence of the types of exchange (numbers in parentheses). This is true even in the case of two-strand doubles, where the potentially good chromatids are twice as numerous as in the case of three-strand doubles, since each tetrad gives rise only to a single gamete. The same principle applies also to triples.

ratio of 3:2; the actual observed small deviation is in the direction expected to result from triple exchanges. The inversion heterozygotes known to give approximately normal crossing over (sc-4, sc-8 and (Df sc^8) actually are the ones that give the higher ratios. The results expected on the pro posed scheme of disjunction in inversion heterozygotes are thus in quantitative agreement with the experimental data.

TABLE 23

Relative frequencies with which double crossovers and patroclinous males are recovered from inversion heterozygotes. In all cases double crossovers as recovered in males only are recorded.

CONSTITUTION	DOUBLE CROSSOVER MALES	PATROCLINOUS MALES	CALCULATED (3:2)		ACTUAL RATIO
sc-4/+	108	63	102.6	68.4	3.4:2
sc-8/+	93	57	90.0	60.0	3.3:2
Df (sc-8)*/+	60	31	54.6	36.4	3.9:2
y-4/+	57	51	64.8	43.2	2.2:2
Df (bb)/+	93	66	95.4	63.6	2.8:2
sc-4/sc-7	11	7	10.8	7.2	3.1:2
	422	275	418.2	278.8	3.1:2

* Number of double crossovers corrected, owing to lethal nature of Df (sc-8).

The scheme proposed should enable one to predict quantitatively the results from closed-X heterozygotes. These have been studied by L. V. MORGAN (1933). Her results differ from those expected according to the scheme formulated from our knowledge of inversion heterozygotes in two important respects:

1. Among the progeny of X/X^c, X is recovered more frequently than X^c.

2. Egg mortality is too high relative to the frequency of recovered double crossovers.

The inequality of X and X^c among the progeny was ascribed by L. V. MORGAN to differential viability. The egg mortality data led her to conclude that single exchanges result in inviable zygotes. Fortunately a second closed-X chromosome was found by Mr. R. D. BOCHE of this laboratory. This closed-X has an advantage over the original X^c used in the experiments of L. V. MORGAN in that it has less effect on viability. We have made several experiments with X^{c-2} heterozygotes set up especially to test the scheme proposed in this paper. The results of these experiments are to be presented in another paper but we can say here that both discrepancies mentioned above appear now to be viability effects. The results obtained from X/X^{c-2} and X^{c-2}/X^{c-2} females are in as good agreement with those predicted from the assumptions we have made in this paper as could reasonably be expected.

We have pointed out that single exchange between two segments inverted relative to one another does not result in inviable zygotes. In the case of attached-X inversion heterozygotes we have pointed out that following certain types of single exchange a chromatid tie is formed during the second meiotic division. From the numerical data we concluded that the condition in which the X chromosome spindle attachment is tied to a spindle attachment in the nucleus lying next in line does result in an inviable egg. There is another case in which single exchange within the inverted segment should result in a chromatid tie at the second division. This is where a single exchange within the inversion is accompanied by a second exchange outside the inversion of such a nature that the two exchanges make a three-strand double exchange. SMITH (1935) has observed this result cytologically in Trillium. In the inversions dealt with in our experiments, lethal eggs from this source would be very infrequent except for the case of In sc-7. This inversion is the only one that we have used in which exchange in the heterozygote is frequent both inside and outside the inverted segment. Here, however, the mortality has not been studied.

EFFECTS OF INVERSIONS ON FREQUENCY OF CROSSING OVER

a. Homozygous inversions. The data are in agreement with earlier conclusions (STURTEVANT 1931) that homozygous inversions show about the same total amount of crossing over as do homozygous normals. They are also in agreement with the conclusions of BEADLE (1932) and of OFFERMAN and MULLER (1932) that the distribution of this crossing over is altered by relation to the spindle attachment, a given section giving less crossing over if it is near the attachment, more if it is near the free end of the chromosome.

b. Heterozygous inversions. The effects on crossing over are, as might be expected, different for sections within the inversion and those outside it, and are also dependent on the length and position of the inversion concerned.

Crossing over within the inversion is evidently decreased in heterozygotes for In sc-7 and In dl-49 (pp. 586–588), and is probably decreased in In ClB since so few doubles are recovered. The other inversions studied here, all of them longer than these, seem to have much less effect on crossing over within the inversion, though the data are scarcely adequate to permit the conclusion that there is no effect.

Crossing over in sections outside the inversion is regularly reduced. In sc-4 and In sc-8 do not leave any sections uninverted in which crossing over occurs in normal flies and can be measured in inversion heterozygotes. The same is true for the sections to the left of In sc-7 and In y-4 and for that to the right of In Df (bb). The remaining seven uninverted

sections all show a reduction in crossing over, localized close to the inversion itself to the right of In sc-7, relatively slight to the right of In dl-49, and very marked in the other cases, namely, on both sides of In ClB, to the left of In dl-49 and of In Df (bb), and to the right of In y-4.

These data are in approximate agreement with expectations from the competitive pairing hypothesis of DOBZHANSKY (1931). A short inversion may be supposed to have its pairing more interfered with by the uninverted sections than does a long inversion which has shorter uninverted sections. Conversely, a long inversion may be supposed to interfere more seriously than a short one with the pairing of the uninverted sections.

One other relation is suggested here, as it is by the data on autosomal inversions (STURTEVANT 1931); namely that an inversion is more effective in suppressing crossing over in segments distal to itself than in proximal segments. This relation is difficult to analyze, and of the present series of inversions dl-49 seems to be the only one favorable for its study. What is needed is a comparative study of a larger series of more diverse types of inversions than we have used. This need is supplied in part by STONE and THOMAS (1935), who reach conclusions similar to the one just suggested.

THE EFFECTS OF THE Y CHROMOSOME ON CROSSING OVER

The most extensive series of data on the effects of a Y chromosome on crossing over is that of BRIDGES and OLBRYCHT (1926). DR. BRIDGES informs us that the XXY females there recorded gave, in addition to the published results, 246 exceptional offspring (2.5 per cent). Correcting the data (by adding twice the number of exceptions to the non-crossover class in the case of XXY females) gives the following comparisons:

	sc	*ec*	*cv*	*cl*	*v*	*g*	*f*	total	N
XX	6.7	8.8	8.2	14.4	11.3	12.3		54.5	11325
XXY	6.5	9.1	8.3	14.2	10.1	9.7		50.8	9461
XX/XXY	1.03	.97	.99	1.01	1.12	1.27		1.07	

Evidently the Y has no effect on crossing over in the region from *sc* to *v*, reduces *v-g*, and reduces *g-f* still more. This is in agreement with the results on duplications described by DOBZHANSKY (1934), since the Y is homologous only with the right end of the X, and reduces crossing over only in the portions of the X near this homologous section.

The results recorded in this paper for comparable XX and XXY females can also be interpreted in terms of the hypothesis of "competitive pairing" (DOBZHANSKY 1931). In Df(bb)/+ gives no effect of the Y, as might be expected, since Df(bb) presumably carries little or no material homologous to the Y. In the cases of sc-4/+ and y-4/+ there is an increase of double

crossing over within the inversion, which may be looked upon as due to interference of the Y with pairing of the attachment ends of the X's, this in turn leading to less interference of these attachment ends with full pairing of the inverted segment. In the case of dl-49/+ the crossing over studied is that between the inversion and the spindle attachment; the data are in agreement with the analysis just given in that they indicate a decrease in crossing over due to the Y. The one remaining case in which we have comparable data is that of y-4/Df(bb), where the frequency of singles within the common inverted region seems to be unaffected by the presence of a Y, as would have been expected.

SECONDARY NON-DISJUNCTION

XXY females of all kinds give segregation of two chromosomes to one pole, one to the other (X-XY or XX-Y). The inversions affect the relative frequencies of these two types, and therefore a fuller understanding of the meiotic behavior of inversions should throw light on the mechanism of secondary non-disjunction.

If p be taken as the frequency of XX-Y segregation, then assuming random fertilization by X sperm and Y sperm and death of the XXX and YY classes, the frequency of recovered exceptions, q, will be $\frac{p}{2-p}$.

The earlier analyses of secondary non-disjunction (BRIDGES 1916, ANDERSON 1929, GERSHENSON 1935) have been based on the assumption that the maximum frequency of XX-Y segregation occurred when one X separated from Y and the other X went to either pole at random. This gives $p=.5$, $q=.333\%$. GERSHENSON himself obtained, from In ClB/+, $q=.353\pm .0040$ in the female classes, a deviation about five times the probable error. Adding our data the value becomes $.366\pm.0038$, a deviation of .033 or nearly 9 times the probable error. In the case of In dl-49/+ the female data of table 14 give $q=.456\pm.0051$, a deviation of .123, 24 times the probable error.

There can be no doubt, then, that XX-Y segregation can occur with a frequency greater than 0.5. As a matter of fact the dl-49/+ value for q (.456) gives $p=.626$.

It becomes necessary, therefore, to search for a new interpretation of secondary exceptions. As pointed out by Bridges (1916), nearly all the exceptional females from +/+/Y mothers are non-crossovers, carrying the same two X's as their respective mothers. The same relation holds for In/+/Y exceptions, as shown by GERSHENSON (1935) and by our own data. In both types of experiments occasional exceptions are found with crossover chromosomes; but these are little, if any, more frequent than are

such crossover exceptions from XX mothers; they may safely be disregarded in analyzing the effect of the Y on non-disjunction. Secondary exceptions, then, carry two unlike non-crossover chromatids. The failure of separation must take place at the first meiotic division, rather than the second, since the latter would give two *like* chromosomes. This is true provided Drosophila agrees with plants in having the first division reductional for spindle attachments. Indirect evidence as well as direct cytological evidence (KAUFMANN, 1935) indicates that this assumption is correct. It may be concluded also that secondary exceptions result from X-*tetrads* in which no crossing over occurred, for otherwise one would have to assume that the orientation of sister chromatids on the second meiotic spindle was not random. This is contrary to what is known in other cases; and even this assumption would not suffice to account for 3-strand or 4-strand doubles.

If secondary exceptions arise from non-crossover X tetrads, the next step is to determine the frequency of such tetrads in various kinds of females and to compare this with the frequency of non-disjunction in such females. This can be done best in the case of In dl-49. As shown above, XXY dl-49/+ females gave about 1 percent exchange to the left of the inversion, 12 percent in the inversion, and 20 percent to the right of the inversion. There are probably not over 3 percent multiple crossovers here, so 30 percent is not far from the true value for the crossover X-tetrads. (Three-strand multiples where one crossover is in the inversion and one is outside it should give rise to inviable eggs; the data presented above show that these are negligible in frequency.) Therefore, among the 70 percent non-crossover X tetrads, 62.6 or 90 percent give rise to non-disjunctional gametes. If we use the frequency of exceptional females actually observed in the same experiment in which crossing over was studied, we find that 66/79 or 83 percent of the non-crossover X tetrads gave XX-Y segregation. This does not take into account exchanges within the inversion. There can be little doubt that 90 percent is too low rather than too high a value. If one assumes that this same proportion holds in all cases the resulting deduced frequencies of complete non-crossover X tetrads (for example 9 percent for +/+/Y) seem not unreasonable. In any case, the proportion .667 suggested by BEADLE and STURTEVANT (1935) by analogy with the fourth chromosome is clearly incorrect for dl-49/+.

Table 14 shows that the frequency of secondary exceptions rises as the total frequency of crossing over decreases in the various inversion combinations. Changes in the reverse direction have not been recorded in *D. melanogaster*, but other species give more crossing over; that is, they have longer crossing over maps and presumably fewer non-crossover tetrads. The available data are shown in table 24. The map lengths given are

probably too short, especially in *willistoni* and *pseudoobscura*, owing to fewness of available loci for study. Other species have been omitted because this element of uncertainty is even greater. It is clear that the table is in agreement with expectation.

TABLE 24

Comparison of species.

SPECIES	SECONDARY EXCEPTIONS		TOTAL MAP LENGTH OF X	
	%	AUTHORITY	UNITS	AUTHORITY
melanogaster	4.3	Bridges 1916	66	Bridges, unpubl.
simulans	2.9	Sturtevant 1929	70	Sturtevant 1929
willistoni	1.7	Lancefield and Metz 1921	84	Lancefield and Metz 1922
virilis	0.5	Kikkawa 1932	182	Kikkawa 1932
pseudoobscura	0	Schultz and Redfield, unpubl.	170	Lancefield 1922

The frequency of secondary exceptions thus shows strong negative correlation with the frequency of tetrad crossing over. Since the latter value is not greatly affected by the presence of a Y, whereas the former is, it may be concluded that the frequency of secondaries is dependent on the occurrence of non-crossover tetrads, rather than the reverse.

NORMAL DISJUNCTION OF X'S

In the case of dl-49/+ the data show about 14 percent crossing over (28 percent exchange) between the spindle attachment and the inversion. The attached-X data show about 12 percent exchange within the inversion. These results are from XXY females; in the cases of other inversions the presence of a Y has been shown to give a slight increase in crossing over within the inversion. There is a small percentage (about 1 percent) of exchange between the inversion and the free end. The indicated total frequency of exchange is thus 41 percent. There is a fairly large probable error attached to this value; but, since there are probably some double exchanges involved, it seems safe to conclude that at least half the tetrads undergo no exchange.

The data of table 14 show, from this combination, no matroclinous females in a total of 3238 daughters. It follows that exchange is not necessary for normal disjunction. This conclusion can be avoided only by supposing that undetectable exchanges occur between the known genes and the attachment end of the chromosome. This supposition has no evidence in its support and is made unlikely by the absence of matroclinous females from In Df(bb)/+ and their presence only in numbers similar to those given by +/+ in the cases of In sc-8/+ and In Df(sc-8)/+. These three cases

all involve inversions that upset the homology well within the inert region, and might well be expected to interfere with crossing over in the region concerned.

The results here reported may, then, be taken as supporting the conclusion, which is probable on other grounds, that crossing over is not a necessary requirement for regular disjunction of the X chromosomes of *Drosophila melanogaster*.

POPULATION MECHANICS OF INVERSIONS

The scheme for inversion crossovers here developed should apply in all cases of inversions that do not include spindle attachments, since singles within such inversions should always give ties between first meiotic nuclei. The resulting selective eliminations of crossover chromatids may be expected in any case where three of the four products of meiosis are eliminated, the effective one being terminal (in terms of the orientation of the second division spindles). These conditions hold in the oögenesis of most animals and in the megasporogenesis of most seed-plants. In such forms as the Ascomycete Neurospora, where all the products of meiosis are potentially functional but are still arranged in a line, inversions of the type under discussion should lead to non-functioning of "inner" nuclei in much higher proportions than terminal ones. In plants the result will be the production of numerous inviable pollen grains, but no increase in egg inviability. There will therefore be no decrease in fertility, a circumstance that must prevent the rapid elimination of inversions through a reduced rate of reproduction. In animals the aberrant sperm will presumably be viable and functional, but will lead to the production of inviable zygotes and therefore to reduced fertility. In Drosophila this result is not brought about because of the absence of crossing over in the male.

A mechanism that increases the number of gametes carrying a single complete haploid set of chromosomes exists also in the case of heterozygotes for reciprocal translocations, where there is a higher frequency of "regular" than of "irregular" gametes in most cases. Here, however, there is no marked sexual difference, and the frequency of irregulars is high enough to produce an appreciable decrease in fertility in most (probably in all) cases. These relations are probably responsible for the observation that, within a given species of Drosophila, wild populations carry inversions far more frequently than translocations.

Inversions that include the spindle attachment cannot produce a chromatid tie, and will therefore decrease fertility if single exchanges occur within them. This is probably the explanation of the fact that no such inversions have been found in wild populations of Drosophila, though they do occur as a result of X-ray treatment.

SUMMARY

1. Seven inversions are discussed. Their nature is illustrated in figure 1.

2. The results obtained from females heterozygous for two inversions are described. The properties of the chromosomes produced by single crossing over within the common inverted sections are summarized in table 11.

3. The frequencies of matroclinous females and patroclinous males from the combinations studied are shown in table 14.

4. Females carrying attached X's, in one of which there is an inversion, give rise to closed X's by single crossing over within the inversion.

5. Egg counts show that the mortality from inversion heterozygotes can all be accounted for as due to the fertilization of no-X eggs by Y sperm. This is very much less than the indicated frequency of single crossover chromatids.

6. Since single crossovers are produced but are not recovered, they must be eliminated from the egg at meiosis, leaving a non-crossover chromatid in the reduced egg.

7. A scheme for such oriented divisions is shown in figures 6 and 7. This is based on cytological observations on plants and on the observed geometrical relations of the meiotic divisions in the Drosophila egg.

8. According to this scheme, crossover chromatids with two spindle attachments form ties between two nuclei at the first meiotic division, resulting in the tied chromatid failing to pass to either terminal pole; or at the second division, resulting in death of the egg when the egg nucleus is concerned.

9. This scheme results in several numerical predictions, which are borne out by the data:

(a) matroclinous females from XX mothers are not increased in frequency by inversions.

(b) patroclinous males are to recovered double crossover males as 2:3.

(c) egg mortality is practically equal to the frequency of patroclinous males.

10. Inversions, and also the presence of a Y chromosome, decrease crossing over in accordance with the hypothesis of competitive pairing.

11. Females (XX) heterozygous for inversions may give many no-exchange tetrads; these segregate normally, with the production of no significant number of XX gametes.

12. In XXY females that are dl-49/+, 90 percent or more of the eggs in which no exchange occurs give XX-Y segregation. Similar frequencies are probable in all cases studied.

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CHROMOSOME STRUCTURE X. AN X-RAY EXPERIMENT†

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INTRODUCTION

IT WAS recently found (NEBEL and RUTTLE, in press) that *Tradescantia reflexa* Raf. shows four threads[1] per chromosome during all somatic as well as during all premeiotic stages.

Multiplication of threads occurs at metaphase during somatic division (fig. 1). In meiosis of *T. reflexa* the four threads multiply at early interkinesis and do not do so again until the metaphase of the first pollen division (fig. 2). The leptotene thread is four-partite. This corresponds to the four-partite structure of chromosomes in mitotic prophases. The chromosome at interkinesis contains eight threads per univalent while at the quartet stage each chromosome again contains four threads. The orientation of these threads disregarding the coils is equidistant for only a short time during telophase. During the remainder of the cycle the chromosome consists essentially of two well-defined chromatids which can only with special precautions be resolved into longitudinal half-chromatids. This orientation is represented by figures 1 and 2.

To demonstrate the quadripartite nature of the presynaptic or leptotene chromosome X-rays were used in order to determine whether such treatment might bring about lesions affecting only one of the constituent threads of a chromosome. If it is possible to influence the discrete threads individually, the cytological study of their subsequent behavior may well complete the demonstration of the quadripartite nature of the presynaptic chromosome. These lesions, while related to the peculiar spatial and functional order of the four threads in a chromosome, seemed to vary in type with variation in dosage, as will be explained below.

LITERATURE AND PRELIMINARY DISCUSSION

X-rays have been used to determine the number of chromonemata in chromosomes by several investigators. According to the majority the chromosome is bipartite during most stages of the cycle. The present find-

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[1] The term "thread" will be used as a morphological term for such ultimate filamentous constituents of the chromosome as can be resolved optically. The term *chromonema* is avoided because it has recently been confused with chromatid. *Genonema* carries theoretical implications and should be reserved for the time when the physical structure of the gene will have been more fully resolved.

ing that the chromosome is four-partite may perhaps be harmonized with that of other investigators under the following premises:

(a) Tradescantia is favorable material with large chromosomes; other material such as Drosophila may have chromosomes which cannot be divided into four parts by either optical or genetical means.

(b) Most workers raying liliaceous plants have not recognized four-partite chromosomes as a normal phenomenon and have therefore not been looking for results in their experiments which might indicate their presence.

(c) Half-chromatids are broken only under certain conditions of dosage and at certain stages. Whether they can be broken in mitosis as well as in meiosis is not certain.

LEWITZKY and ARARATIAN (1931), radiating somatic cells of Secale, found after 2 days a large number of fragments some of which were very small. These small fragments they believe may have come from parts of the chromosome which are naturally attenuated (the kinetochore and its neighborhood). According to the present view they may come from fragmentation of one of the four constituent threads of the chromosome. STONE (1933) and MATHER and STONE (1933), raying somatic tissue of liliaceous plants, observed only breaks of entire chromosomes. MATHER (1934) rayed cymes of Tradescantia and observed the resulting chromosome changes from 3 to 24 days after the treatment. Although MATHER did not use methods which would show the threads plainly, his figure 18, showing first pollen grain divisions nine days after radiation, indicates that the chromosome is four-partite before meiosis. SAX and EDMONDS (1933) give a time schedule for the development of the pollen grains in Tradescantia, according to which the material which MATHER shows in his figure 18 was apparently rayed before synapsis. Small fragments of about half the diameter of a chromatid are also present in figure 11 of HUSKINS and HUNTER (1935), who radiated microspores of Trillium after the second reduction division and observed the first division in the pollen grain. These results indicate that breaks in half-chromatids may be observed in radiation of nuclei of the somatic type. MARSHAK (1935) concludes from his work on Gasteria that the chromosome previous to synapsis is "at least two-partite."

According to earlier findings (NEBEL and RUTTLE in press), the chromosome in Tradescantia is visibly four-partite from the last premeiotic metaphase until early interkinesis (fig. 2). Each constituent thread then multiplies, the eight resulting threads forming sister chromosomes of the second meiotic division. The next multiplication of threads occurs at metaphase of the first division of the pollen grain. Any normal chromosome in a metaphase plate of the first division in the pollen grain is thus the

direct descendant of one chromatid of pre-meiosis. Any surviving fragments of half-chromatids produced at pre-meiosis would, if they divided normally, appear as longitudinal half-chromosome (chromatid) fragments in the early metaphase of the first division of the pollen grain. When the original fragments survive but are too small to be capable of division, they will show up in the pollen grain division as minute bodies of about one-fourth the diameter of a normal chromosome.

The observations of LEVITZKY and ARARATIAN (1931) on somatic cells of rye can be interpreted according to the present view point. The small fragments shown in their figure 2 plate 1 from divisions fixed two days after radiation may be fragments of half-chromatids. The authors vainly seek an explanation of these very small fragments. MATHER (1934) also shows small fragments in his figure 18. These, as well as the large chromosome pieces which consist of only one chromatid of prometaphase of the pollen grain division, are interpreted to be the direct descendants of breaks of half-chromatids, which occurred during presynapsis. HUSKINS and HUNTER (1935) likewise probably obtain breaks in half-chromatids of Trillium, indicating that the chromosomes of this plant are four-partite at all stages. MARSHAK (1935) considers the chromosome at least two-partite before synapsis. MARSHAK was working with relatively low dosages of X-rays which according to the present results give only a very low frequency of half-chromatid breaks or none at all. For this reason MARSHAK's findings are considered not to contradict the present work. Thus it may be said that the data of the literature while not directly so interpreted by the respective investigators may be in actual agreement with the fact that the chromosomes of many monocots are four-partite at all stages.

This conclusion apparently does not hold for animal material as far as it has been carefully investigated. PATTERSON (1933 and 1935) and MOORE (1935) have shown from genetical evidence that the interphase chromosome of Drosophila is a bipartite structure. Radiation of mature sperm gave complete mutations as well as mosaics. The latter indicate that there are two gene threads per chromosome in the resting stage. Why both threads may be affected identically by radiation will be discussed later. WHITE (1935) rayed spermatogonia of Locusta and observed various types of lesions during the first mitosis, which occurred after treatment. When metaphases were observed 32 hours after radiation WHITE found chromatid breaks, indicating that the chromosome was split in two at the time of radiation. WHITE's data thus indicate that orthopteran chromosomes are physiologically double at all stages of the mitotic cycle.

MATERIALS AND METHODS

Cymes (flowering heads) of plants of *T. reflexa* were radiated with 50, 200, 500 and 1000 *r* units respectively. A Coolidge tube running at 180 k.v.p. and 3 milliamperes furnished the radiation. All buds containing stages later than diakinesis were removed previous to exposure. It is assumed that the majority of the sporocytes subjected to radiation contained presynaptic nuclei for the following reason. In *T. reflexa* reduction division roughly follows a daily cycle, and successively younger buds in a vigorous half-cyme show stages separated by a definite interval. From leptotene to first metaphase requires approximately 24 hours. If all buds later than diakinesis are removed in a given cyme, it is safe to say that all remaining buds are in presynapsis at that time. In less vigorous cymes such as were used in the present experiment, successively younger buds show even greater time intervals.

Smear preparations were made in the following way: pretreated in ½ normal Ringer 30 seconds at pH 7.5; fixed in 3:1 alcohol acetic acid; stained with carmine alum lake in 45 percent acetic acid; dehydrated for two minutes each in alcohol acetic acid 2:1, 4:1, 10:1, absolute alcohol, clove oil; mounted in diaphane. This method yields slides which will keep for a few months. The use of clove oil was suggested by M. L. RUTTLE (unpublished). Had it not been for this method the half-chromatid breaks described in this paper would undoubtedly have escaped observation.

OBSERVATIONS

The microscopic evidence of half-chromatid and chromatid lesions of presynapsis is shown in figure 3, A–D. These chromosomes are from cells radiated during presynapsis; A, B and C are from second anaphase, D is from prometaphase of the first division in the pollen grain. The chromosome of second anaphase contains four threads, two in each chromatid. In figure 3, A and B there are visible asymmetries between sister chromatids. Since each chromatid of the second anaphase is the equivalent of a half-chromatid of the cycle preceding first metaphase, lesions as shown in figure 3, A and B are termed half-chromatid lesions of presynapsis. Figure 3C shows an entire chromosome of second anaphase, which forms a slight angle at the insertion region. On the vertical arm there are two achromatic spots on sister chromatids which are due to a lesion of a presynaptic chromatid. Figure 3D is from prometaphase of the first division in the pollen grain. The chromosome is obviously split at this stage into the two prospective chromosomes of anaphase and each half contains two threads which are about to become four. The asymmetric pieces towards 10 and 2 o'clock are prospective anaphase chromosomes which have no sister halves. The

asymmetry of prometaphase chromosomes of the first division in the pollen grain corresponds to lesions which affected half-chromatids of presynapsis. With reference to figure 3, A and B it may be said that they are presented as examples of a phenomenon which appeared only under the specific conditions of radiation of 200 *r* units and above. The origin of each single case might be questioned but the occurrence of this type of lesion exclusively under the condition of the experiment renders the individual case significant.

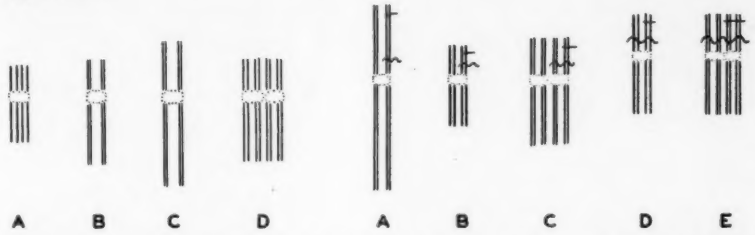

FIGURE 1.—Diagram of Mitosis. A, telophase with threads equidistant. B, interphase. C, prophase. D, metaphase. Multiplication of threads becomes visible during metaphase as soon as the prospective chromosomes of anaphase are ready to separate. Coiling has been disregarded; also the threads are not actually arranged in a single plane.

FIGURE 2.—Diagram of number of threads in a single chromosome in meiosis and metaphase of first division in the pollen grain. A, leptotene. B, first metaphase. C, interkinesis. At this stage multiplication of threads becomes visible; each chromosome contains eight threads which separate at the second division. D, quartet stage (only one of the two chromosomes which separate in the second division is shown). E, metaphase in first division of the pollen grain. Another multiplication occurs here. "Lesions" have been entered by cross bars, the straight horizontal bar illustrates the history of a lesion affecting a half chromatid of presynapsis. The wavy cross bar illustrates a lesion affecting one chromatid of presynapsis. A half-chromatid lesion of presynapsis becomes a chromatid lesion at second anaphase and a chromosome lesion after the metaphase of the first division in the pollen grain. A chromatid lesion of presynapsis becomes a chromosome lesion at second division etc.

Table 1 is a summary of the observations. The effect of radiation was observed primarily during first and second reduction division. Turning to figure 2, it can be seen that a lesion affecting one chromatid at leptotene (fig. 2A) would be observed as such during first division, (fig. 2B) but would during late metaphase and anaphase of the second division appear as a lesion affecting a chromosome (fig. 2D). At the division in the pollen grain this same lesion would affect both halves of the chromosome at prometaphase (fig. 2E).

TABLE 1

Effect of X-rays on presynaptic chromosomes observed during first and second anaphase

DOSAGE r UNITS	MEIOTIC DIVISION	HOURS AFTER TREATMENT	TOTAL LESIONS	COUNTS OF LESIONS AFFECTING				PERCENTAGE OF LESIONS AFFECTING			
				BOTH HOMOLOGUES	ONE CHROMOSOME	ONE CHROMATID	1 HALF-CHROMATID	BOTH HOMOLOGUES	ONE CHROMOSOME	ONE CHROMATID	1 HALF-CHROMATID
1000	2nd	60.5	81	2	18	51	10	2.5	22	63	12.3
500	1st	53	46		2	42	2		4.4	91.2	4.4
	2nd	22	183		5	157	21		2.7	85.7	11.6
200	1st	26	35		2	32	1		5.7	91.4	2.9
	2nd	26	120		9	109	2		7.5	90.8	1.7
50	1st	29	55			55				100	
	2nd	29	32		1	32			3	97	

A lesion affecting one half-chromatid at leptotene becomes a lesion of half a chromosome in second division and of a whole chromosome in the anaphase of the division in the pollen grain. In table 1 observations made

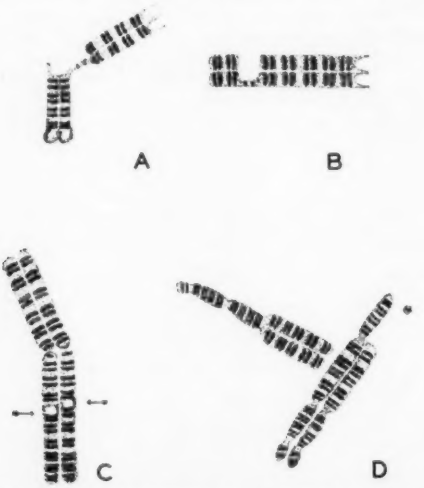

FIGURE 3.—Material radiated during presynapsis. A, B and C are from second anaphase, A, showing one chromatid asymmetrically attached to the end of another chromosome, B, showing one chromatid partly deleted. Half-chromosome (chromatid) lesions of second anaphase are the result of half-chromatid lesions of presynapsis. C illustrates two achromatic spots at the same level of a chromosome at second anaphase. These are the results of a lesion affecting one chromatid of presynapsis. D is from prometaphase of the first division in the pollen grain. The asymmetric pieces towards 10 and 2 o'clock are prospective anaphasic elements. Their sister halves have become detached. This corresponds to half-chromatid lesions of presynapsis. Camera lucida ×2400. (Drawn by M. L. Ruttle.)

on first and second divisions are reduced to the condition of the chromosome at first metaphase, which in Tradescantia is identical with the condition in leptotene or any other presynaptic stage after the last archesporial division. Thus if at second anaphase a lesion is observed which at that stage involves a single chromatid, this is entered in table 1 as a lesion having affected one half-chromatid.

The term lesion is used to include any type of cytological abnormality, such as achromatic spots, breaks or translocations, here again using the last term to describe the transfer of chromosomes or parts of chromosomes to previously unrelated parts of other chromosomes.

A lesion which affected a half-chromatid observed in second division could not be distinguished from a lesion affecting three half-chromatids. Breaks affecting three half-chromatids at first division were, however, not observed. Achromatic spots affecting a half-chromatid only were not observed. Achromatic spots seemed always to affect both halves of a chromatid (fig. 3c) and appeared in second division side by side as a clearly defined pair of spots or as narrow achromatic band across the width of a chromosome of second anaphase.

The data in table 1 seem to indicate that the effect of radiation changes with increasing dosage. At low dosages the effect is confined almost completely to lesions of chromatids. At higher dosages there is an increasing number of other lesions. Single half-chromatid lesions increase and lesions involving more than one chromatid at a given level become more numerous.

Since higher dosages of radiation merely represent longer exposures to the same type of radiation it is hard to understand why at the low dosage the types of lesions were narrowed to nearly a single class.

To explain the results it is suggested that under longer exposures the reaction of the chromatic elements is no longer a simple one. Under prolonged exposure heat may be generated in the cell, sensitive processes of metabolism may be invalidated sufficiently to change the reaction of chromatin toward radiation. In a general sense prolonged radiation causes a change in the environment of the chromosome threads, which in turn changes the type of reaction of the chromosomes toward radiation. Under low dosage, with no change in the environment, the unit of reaction within the chromosome is the chromatid. This is in keeping with the fact that crossing over is a reaction between chromatids, not between half-chromatids. It is also in keeping with the observation that half-chromatids are very closely united, perhaps by a common matrix.[2] It is assumed that

[2] I have suggested earlier (NEBEL 1932) that each chromonema is surrounded by its own individual matrix. In a general sense this is still maintained. If the diameter of the gene string (HASKINS 1935) is a fraction of that of a visible chromosome thread, this visible surrounding of

sister half-chromatids form a physiological unit, which under moderate radiation reacts as a unit. Under increased radiation, due to a change in the environment the equilibrium between half-chromatids may become upset, and the normal physiological and spatial distance between related chromatids and chromosomes may also become disturbed. The effect of heavy radiation is similar to that of heat which causes clumping and sticking of chromatic elements which normally form no contacts.

The present evidence is purely cytological but chromatic changes may entail genetic changes. The finding of STUBBE (1935) that the genom of *Epilobium hirsutum* is more sensitive to radiation when carried in *E. luteum* plasma than when carried in its own plasma may be quoted as an analogy for the instability of the genom under changes of environment.

The existence of half-chromatid breaks is not taken as proof but merely as a confirmation of the morphological observation that meiotic chromatids are formed and split in the last premeiotic metaphase in Tradescantia. It would be possible to explain the data differently but any alternative explanation leads into additional difficulties.

MARSHAK (1935) found a linear progression for lesions with dosage. The present material confirms MARSHAK'S finding. A special study of this phase was not possible but when a graph was drawn from the number of lesions observed under various dosages using only those cells in which all chromosomes could be seen, this graph showed 2 to 3 lesions per cell with 50 *r* units, 4 to 5 with 200, 8 to 10 with 500 and 10 to 18 with 1000 *r* units respectively. The graph is not given here because the numbers of perfect cells available for counting was low. Lesions involving a chromatid were counted as one, lesions involving a half-chromatid likewise. Lesions involving more than one chromatid were counted as one only where sister chromatids clearly appeared involved at the same level.

With higher dosages counts of lesions will appear low, for the reason that if a fragment becomes a micronucleus it will hide additional lesions which it may contain. Counts of lesions will appear high for the following reason. It is assumed that a single hit may at higher dosage spread "horizontally" —at right angles to the long axis of the chromosome. The first division separates homologous chromatids and the second division separates sister

the gene string may be called a matrix. Other cytologists (KUWADA and NAKAMURA 1934) have postulated several matrices within the chromosome to account for the different coils which may be formed at one time within the chromosome. It may be well to maintain the term matrix and think of various matrices one inside the other, but these terms are merely descriptive images of what actually is effected by various forces of mutual attraction between adjacent threads. In the present case we must postulate one matrix to keep the four threads of a chromosome together. The second matrices hold chromatids at the correct distance and surround half-chromatids. The third matrices surround half-chromatids in Tradescantia. All three matrices are to our knowledge identical in substance.

chromatids. This separation makes it hard to detect lesions in corresponding regions of sister and homologous chromatids. The effect of what may have been a single hit will thus be considered multiple.

These two phenomena may cancel one another. With this in mind the obtained linearity of the curve of number of lesions per cell plotted against dosage suggests that under higher dosages the effect of a single hit may occasionally spread at right angles to the longitudinal axis of a chromosome, and also may, in contrast to the effect of low dosages, remain confined to a single half-chromatid. A few observations were made on the first somatic division in the pollen grain from cymes rayed with 500 *r* units. The divisions were observed 14 days after radiation. This time interval indicates that at the time of radiation the material was in presynapsis. Several divisions showed an abundance of fragments involving only one chromatid of prometaphase. From diagrams A to E of figure 2 it may be seen that such breaks would be expected from the lesion of half-chromatids of presynapsis.

The occurrence of half-chromatid breaks as observed at first and second division was followed thru successive days and could be traced for about a week after treatment. After this time half-chromatid breaks were no longer observed. This one would expect if the maximum time limit between reduction division and the last preceding archesporial division for any one cell were around 170 hours. This corresponds to the time schedule given by SAX and EDMONDS (1933).

SUMMARY

Radiation of cymes of *Tradescantia reflexa* Raf. was applied to presynaptic stages of microsporocytes. The effect of the radiation was observed during first and second reduction divisions. With low dosage the experiment indicates that the chromosome is split in *two* previous to synapsis. With higher dosage the experiment suggests that occasionally the chromosome is split into *four* previous to synapsis.

This is interpreted as follows: Each chromosome is composed of two split chromatids (or four half-chromatids) at all stages of presynapsis. In normal material the two half chromatids that constitute a given chromatid lie very close to one another and do not react individually. (The process of crossing over in *Tradescantia* is a reaction between chromatids in which every two half-chromatids behave as a physiological and mechanical unit.)

Under low dosage of X-rays half-chromatids are physiologically unable to show separate reactions.

With higher dosage of radiation (200 *r* units and more) the spatial and physiological order of the threads within a chromosome is upset. Chromatic reactions may be recorded which affect one of four (or three of four)

threads differentially. It is also indicated that with higher dosage chromatic reactions at a certain level of the chromosome may spread, involving sister and even homologous loci.

The differential effect of higher dosage is thus attributed to action upon the immediate environment of the chromatin, perhaps to heat or to a general interference with the normal metabolism of the nucleus which in turn changes the type of reaction of the chromatin.

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THE EFFECT OF AUTOSOMAL INVERSIONS ON CROSSING OVER IN THE X-CHROMOSOME OF DROSOPHILA MELANOGASTER

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INTRODUCTION

THE first evidence of a crossover suppressor was found in 1913 by STURTEVANT. In 1921 STURTEVANT found the first definite evidence of an inversion when he showed that several third chromosome genes in *Drosophila melanogaster* were arranged in an inverted order with respect to similar genes in the third chromosome of *D. simulans*. At this time STURTEVANT predicted that a heterozygous inversion would suppress crossing over and suggested that the Nova Scotia stock (STURTEVANT 1917 and 1919) had an inversion. It was not until 1926, however, that it was actually demonstrated that in at least one case crossover suppression was due to an inversion (STURTEVANT 1926).

The sole effect on crossing over ascribed to inversions until recently is that the number of recovered crossovers is reduced in the chromosome in which the inversion is located. But as long ago as 1919, STURTEVANT suspected that crossover suppressors (inversions) might have an interchromosomal effect. He reported that a high value for crossing over between black and purple was due, in part at least, to a dominant gene (really an inversion) in the third chromosome, which when heterozygous reduced crossing over in this chromosome.

WARD (1923) tested the effect of the curly inversion on crossing over in the first and third chromosomes; her observations on crossing over were of no value because of the small number of flies used, but she did notice an increase in multiple crossovers. PAYNE (1924) tested the effect of the Payne inversion on crossing over in the first and second chromosomes. While he counted large numbers of flies, the chromosomes were poorly marked (*w*^{e}*m* on the first chromosome and *b pr c sp* on the second) and he ran no controls; he therefore noticed no interchromosomal effect on crossing over. PAYNE made no statement in regard to the number of multiple crossovers recovered.

The problem rested here until 1932, when SCHULTZ and REDFIELD (MORGAN, BRIDGES and SCHULTZ 1932) stated that inversions in the first and second chromosomes increased crossing over in the third chromosome. In the following year they published a short account (MORGAN, BRIDGES

and SCHULTZ 1933) in which they stated that inversions in the first and third chromosomes increased crossing over in the second. The increased crossing over in both cases was due to an increase in multiple crossing over.

The experiments reported in this paper are concerned with the effects of inversions in the second and third chromosomes on crossing over in the first chromosome.

MATERIALS AND TECHNIQUE

The autosomal inversions used in the experiments were the Payne inversions (C_3LP *Dfd* C_3RP *ca*) in the third chromosome and the Cy inversions (Cy $C_2L.C_2R_{Cy}$) in the second chromosome. The Payne inversions (PAYNE 1924) reduce crossing over almost completely throughout the length of the third chromosome. The Curly inversions (WARD 1923) gave no crossover flies in a total of 2487 from a cross of heterozygous Cy/2ple females by 2ple/2ple males. Crossing over in the first chromosome was detected by means of the alternated X^7 stock (*y cv v f/ec* ct^6 g^2). This is identical with the alternated X-ple stock (BRIDGES and OLBRYCHT 1926) except for the substitution of *y* for *sc*.

The crosses were as follows:

1. +/+; +/+; *y cv v f/ec* ct^6 g^2 ♀ × Oregon-R ♂ (controls)
2. +/+; +/Cy; " / " ♀ × " ♂
3. +/Payne; +/+; " / " ♀ × " ♂
4. +/Payne; +/Cy; " / " ♀ × " ♂

Because of a significant deviation of the crossover value in region 1 of the controls from the standard value (to be discussed below) it is important that the relation of the chromosomes of the test stocks to those of the controls be explained. The derivation of the stocks was such that one set of autosomes and one X chromosome in each stock came from the original *ec* ct^6 g^2 stock. The second X chromosome in each case came from the original *y cv v f* stock. The origin of the remaining chromosomes was as follows: in the controls they all came from the *y cv v f* stock. In the +/+; +/Cy; *y cv v f/ec* ct^6 g^2 stock the remaining third chromosome had an equal chance of coming from either the *y cv v f* or the Curly stock; the same holds true for the fourth chromosome. In the +/Payne; +/+; *y cv v f/ec* ct^6 g^2 stock the remaining second and fourth chromosomes had an equal chance of coming from either the Payne or the *y cv v f* stock. Since twenty cultures with from two to three females in each were counted for each cross, it is likely that the origins of these chromosomes were very nearly equally divided between their possible sources. In the +/Payne; +/Cy; *y cv v f/ec* ct^6 g^2 stock only the origin of the fourth chromosome is in doubt. It had an equal chance of coming from either the Payne, the Curly or the *y cv v f* stock.

In all the crosses the male offspring only were classified for each of the seven first chromosome characters involved. The crosses were run at $25 \pm 1°C$ on the usual cornmeal-agar-molasses food medium.

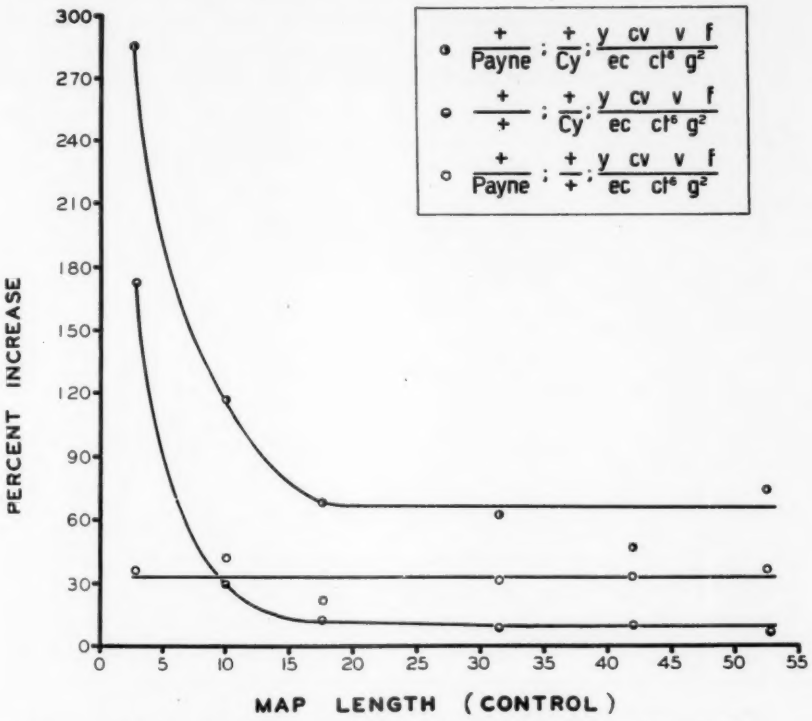

FIGURE 1.—Plot of percent increase in crossing over in the test crosses over that of the controls against the control map length. (Data from table 1.)

THE DATA

The crossover values in the controls for all regions except region 1 compare favorably with the standard map distances (table 1). Region 1, however, gives a value which is but half that of the standard value. Since a large number of flies was counted (3937) and classification was certain, this difference must be considered significant. The construction of the stocks used in the test crosses makes it unlikely that this low value is due to any autosomal modifiers. Since the source of the X chromosomes is constant throughout the experiment it is almost certain that the cause of this low value in region 1 of the controls had no effect on the relative

values of the results, although the possibility remains that there might have been some effect on the absolute values.

Table 1 shows the crossover values for each of the four different crosses as well as a comparison of the crossover values of the test crosses with those of the controls. These values are calculated from the raw data shown in table 5. There is an increase in crossing over in each of the six regions of the test crosses as compared with the controls. This increase is greatest

TABLE 1

Comparison of crossover values.

REGION	1		2		3		4		5		6		MAP LENGTH		TOTAL NUMBER OF FLIES
	% CROSSOVER	% INCREASE	% CROSSOVER	% INCREASE	% CROSSOVER	% INCREASE	% CROSSOVER	% INCREASE	% CROSSOVER	% INCREASE	% CROSSOVER	% INCREASE	MAP LENGTH	% INCREASE	
+ + *y cv v f* / − ; − ; − / + + *ec* ct^6 g^2	2.7	—	7.4	—	7.7	—	13.6	—	10.7	—	10.7	—	52.8	—	3937
+ + *y cv v f* / − ; − ; − / + Cy *ec* ct^6 g^2	7.4	171.1	9.8	32.4	8.7	13.0	14.8	8.8	11.9	11.2	11.5	7.5	64.1	21.4	2733
+ + *y cv v f* / ——— ; − ; ——— / Payne + *ec* ct^6 g^2	3.7	37.0	10.6	43.2	9.2	19.5	17.8	30.9	14.3	33.6	14.8	38.3	70.4	33.3	3612
+ + *y cv v f* / ——— ; − ; ——— / Payne Cy *ec* ct^6 g^2	10.4	285.2	16.1	117.6	12.9	67.5	22.2	63.2	16.0	49.5	19.4	75.4	97.0	83.7	1554

in the cross involving both sets of inversions, next greatest in the cross with the Payne inversions and least in the cross with the Curly inversions (table 1, fig. 1). This is due to a large increase in multiple crossovers which is again greatest in the cross involving both sets of inversions, and least in the cross involving the Curly inversions.

The Curly inversions have their greatest effect on the left end of the X chromosome, causing an increase in crossing over of 171.1 percent between *y* and *ec* (region 1, table 1). The effect drops rapidly as the right end of the chromosome is approached so that between *cv* and ct^6 (region 3, table 1) the increase is only 13.0 percent. From this point on the effect on crossing over is approximately constant (table 1, fig. 1).

The Payne inversions, unlike the Curly inversions, have an approximately uniform effect on crossing over throughout the portion of the X chromosome under observation in this experiment (table 1 and fig. 1). This effect is to cause an increase of approximately 33 percent in each of the six regions. According to SCHULTZ and REDFIELD (MORGAN, BRIDGES and SCHULTZ, 1933), the Payne inversions did not have a uniform effect throughout the length of the second chromosome but affected crossing

over in this chromosome in much the same manner as does triploidy. Why the effect of the Payne inversions on one chromosome is different from their effect on another cannot be explained at present.

The Curly and Payne inversions when combined affect crossing over in the first chromosome in somewhat the same manner as do the Curly inversions alone (table 1 and fig. 1). Here again, the increase is greatest in region 1 (285.2 percent) and decreases rapidly to 65.7 percent in region 3 and remains practically constant thereafter (table 1 and fig. 1). The effect of the combined inversions is greater in every region than is the effect of the inversions taken singly. Furthermore, the effect of the combined inversions exceeds in every region the sum of the effects of each of the two pairs of inversions used separately (table 1).

The increase in crossing over noted in the crosses involving the inversions singly or together is due to an increase in multiple crossover strands and a decrease in non-crossover strands (tables 2, 3 and 4). In the cross

TABLE 2

Classification of strands into crossover classes.

	CONTROLS + + *y cv v f* / + + *ec ct*^{6} *g*^{2}		+ + *y cv v f* / + Cy *ec ct*^{1} *g*^{2}		+ + *y cv v f* / Payne + *ec ct*^{6} *g*^{2}		+ + *y cv v f* / Payne Cy *ec ct*^{6} *g*^{2}	
	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT
non-crossovers	2052	52.1	1213	44.4	1532	42.4	470	30.2
singles	1697	43.1	1292	47.3	1632	45.2	702	45.2
doubles	181	4.6	224	8.2	430	11.9	344	22.1
triples	7	0.2	4	0.1	16	0.4	37	2.4
quadruples	—	—	—	—	2	0.1	—	—
quintuples	—	—	—	—	—	—	1	0.1
totals	3937		2733		3612		1554	

TABLE 3

Showing comparison of tetrads having a crossover in a given region with those having a crossover in the given region and in one other region. All values are in percent.

REGIONS	1 SINGLES	1 DOUBLES	2 SINGLES	2 DOUBLES	3 SINGLES	3 DOUBLES	4 SINGLES	4 DOUBLES	5 SINGLES	5 DOUBLES	6 SINGLES	6 DOUBLES
+ + *y cv v f* / + + *ec ct*^{6} *g*^{2}	2.8	2.3	9.9	4.4	9.8	5.0	19.9	2.8	16.1	5.0	10.4	6.5
+ + *y cv v f* / + Cy *ec ct*^{6} *g*^{2}	6.7	7.7	13.8	8.7	9.2	7.9	17.9	11.1	11.3	11.8	7.3	15.1
+ + *y cv v f* / Payne + *ec ct*^{6} *g*^{2}	2.3	4.6	7.0	11.9	6.8	11.6	14.0	20.1	11.0	15.3	4.4	23.0
+ + *y cv v f* / Payne Cy *ec ct*^{6} *g*^{2}	1.4	11.4	−1.3	26.0	5.9	13.4	6.6	21.0	2.8	21.3	1.0	27.6

with the Curly inversions the double crossover strands are increased by 78 percent (table 2). The non-crossover strands are decreased from 52.1 percent to 44.4 percent, a reduction of 14.8 percent. There is an apparent reduction in the number of triple crossovers but the numbers involved are small and therefore the reduction is probably not significant. The increase

TABLE 4

Distribution of crossovers in tetrads. All values are in percent. Symbols are explained in the text. (The 5 point crossover is not included in the calculations of $T_{.i}$, $T_{.ij}$, etc.; the values of the T_{ijk} tetrads are omitted.)

CLASS	+ + *y cv v f* / + + *ec ct⁸ g²*	+ + *y cv v f* / + Cy *ec ct⁸ g²*	+ + *y cv v f* / Payne + *ec ct⁸ g²*	+ + *y cv v f* / Payne Cy *ec ct⁸ g²*
T_0	13.4	5.2	8.8	4.7
$T_{.i}$	68.9	62.4	44.4	16.4
$T_{.ij}$	16.2	31.6	45.2	59.6
$T_{.ijk}$	1.4	0.8	0.0	19.2
$T_{.ijkl}$	—	—	1.6	—
REGION		BY REGIONS		
1	2.8	6.7	2.3	1.4
2	9.9	13.8	7.0	−1.3
3	9.8	9.2	6.8	5.9
4	19.9	17.9	14.0	6.6
5	16.1	11.3	11.0	2.8
6	10.4	7.3	4.4	1.0
1, 2	−0.1	−0.1	0.0	0.0
1, 3	0.0	0.4	0.0	1.3
1, 4	0.5	2.3	1.9	3.1
1, 5	0.7	2.3	1.5	2.1
1, 6	1.2	2.8	1.2	4.9
2, 3	−0.1	—	0.2	0.3
2, 4	0.7	1.5	2.9	9.0
2, 5	1.2	4.2	3.2	8.2
2, 6	2.6	3.1	5.6	8.5
3, 4	0.7	0.6	2.0	1.8
3, 5	1.5	3.1	4.1	5.1
3, 6	2.8	3.8	5.2	4.9
4, 5	1.3	1.6	4.4	1.8
4, 6	−0.4	4.8	8.9	5.2
5, 6	0.2	0.6	2.1	4.1

in single crossover strands is probably significant. ($D/\sigma_d = 3.5$.) The Payne inversions cause an increase in double crossover strands of 154 percent and a decrease of 18.4 percent in the non-crossover strands. The single crossover strands do not change (43.1 percent in the controls and 45.2 percent in the Payne cross). Here again the numbers of three point crossover strands are too small to give decisive evidence as to the effect on triple crossovers. The combined inversions have a great effect on both the mul-

tiple crossover strands and the non-crossover strands. Double crossover strands are increased by 369 percent and triple crossovers by 1100 percent. The single crossover strands remain constant. The non-crossover strands are decreased by 41.7 percent (52.1 percent in the controls and 30.2 percent when both the Curly and Payne inversions are present).

The behavior of the tetrads in all four crosses was deduced by means of the following formulae for the distribution of crossovers in tetrads. (These have been derived by D. R. CHARLES [unpublished] from WEINSTEIN'S generalized tetrad formulae [WEINSTEIN 1932 and 1936].)

$$T_{.ijkl} = 16s_{.ijkl}$$
$$T_{.ijk} = 8(s_{.ijk} - 4s_{.ijkl})$$
$$T_{.ij} = 4(s_{.ij} - 3s_{.ijk} + 6s_{.ijkl})$$
$$T_{.i} = 2(s_{.i} - 2s_{.ij} + 3s_{.ijk} - 4s_{.ijkl})$$
$$T_{.o} = s_{.o} - s_{.i} + s_{.ij} - s_{.ijk} + s_{.ijkl}$$

Where $T_{.ijkl}$ = all tetrads with four crossovers regardless of the regions involved; $T_{.ijk}$ = all tetrads with three crossovers regardless of the regions involved etc. and where $s_{.ijkl}$ = all strands with four crossovers regardless of the regions involved etc.

Distribution of tetrads with crossovers in specific regions:

$$T_{ijkl} = 16s_{ijkl.}$$
$$T_{ijk.} = 8(s_{ijk.} - s_{ijk.l})$$
$$T_{ij.} = 4(s_{ij.} - s_{ij.k} + s_{ij.kl})$$
$$T_{i.} = 2(s_{i.} - s_{i.j} + 3s_{i.jk} - s_{i.jkl})$$

Where $T_{ijkl.}$ = all tetrads which are crossovers in the specific regions ijkl only and $T_{ijk.}$ = all tetrads which are crossovers in the specific regions ijk only, etc., and $T_{ijk.l}$ = all tetrads which are crossovers in the specific regions ijk and one other region, l, and where $s_{ijkl.}$, $s_{ijk.}$, etc., have the same meaning for strands as $T_{ijkl.}$, etc., has for tetrads.

The tetrad computations of the data from the Curly cross indicate that those tetrads which show no crossovers in the portion of the X chromosome under observation (T_o in table 4) are greatly reduced in number and those tetrads which show one crossover ($T_{.i}$ in table 4) are slightly reduced in number as compared with the controls. The numbers of tetrads showing two crossovers is greatly increased. In the cross with the Payne inversions, the decrease in non-crossover tetrads is not as great as that in the cross with the Curly inversions, but the effect on the single crossover and double crossover tetrads, while in the same direction as that caused by the Curly inversions, is far greater. As is to be expected, the combined inversions have a much greater effect than either of the inversions alone. They reduce the non-crossover tetrads to 4.7 percent of the total and single crossover

tetrads to 16.4 percent while they increase the double crossover tetrads to 59.6 percent and the triple crossover tetrads to 19.2 percent.

Table 3 shows a comparison of tetrads having a crossover in a given region with those having a crossover in the given region plus one other region. In the controls we find that most of the crossing over in all six regions comes from single crossover tetrads (tetrads having only one crossover, that crossover being in the given region). In the cross with the Curly inversions, in regions one and six most of the crossing over occurred in $T_{i.j}$ tetrads (tetrads with two crossovers, one of which is in the given region i). In region 5 about the same amount of crossing over occurred in $T_{i.}$ tetrads as in $T_{i.j}$ tetrads. In the remaining regions most of the crossing over took place in $T_{i.}$ tetrads. In the cross involving the Payne inversions, most of the crossing over took place in the $T_{i.j}$ tetrads. This is the reverse of what took place in the controls. Finally, in the cross with the combined inversions, not only did most of the crossing over take place in $T_{i.j}$ tetrads, but these tetrads exceeded the $T_{i.}$ tetrads by at least 100 percent in every region. In regions 1, 2, and 6 the excess of $T_{i.j}$ tetrads over $T_{i.}$ tetrads is tremendous (table 3). Note the great reduction in the number of tetrads which crossed over in region 2 only.

TABLE 5

Showing the numbers and types of ♂ offspring of each of the four different classes of ♀ ♀ listed below.

REGIONS	+ + *y cv v f* / + + *ec ct⁶ g²*		+ + *y cv v f* / + Cy *ec ct⁶ g²*		+ + *y cv v f* / Payne + *ec ct⁶ g²*		+ + *y cv v f* / Payne Cy *ec ct⁶ g²*	
	y	*non-y*	*y*	*non-y*	*y*	*non-y*	*y*	*non-y*
0	1033	1019	640	573	861	671	254	216
1	44	35	59	87	39	46	21	50
2	118	122	102	99	122	123	67	40
3	137	107	96	85	109	118	52	59
4	228	231	161	161	230	208	76	90
5	173	194	124	113	186	156	58	58
6	145	163	97	108	171	124	81	50
1, 2	0	0	0	0	0	1	1	1
1, 3	0	1	0	3	0	0	3	5
1, 4	2	3	8	9	6	12	10	12
1, 5	2	5	8	8	5	10	6	12
1, 6	6	6	10	9	6	6	10	15
2, 3	0	0	0	0	1	1	1	2
2, 4	4	4	8	5	15	14	24	22
2, 5	7	6	20	10	16	23	24	13
2, 6	13	15	16	6	26	29	23	18
3, 4	4	5	4	0	9	9	8	8
3, 5	10	5	13	9	22	16	11	13
3, 6	15	15	13	14	19	29	14	12
4, 5	7	7	6	6	22	22	13	8

TABLE 5 (*Continued*)

Showing the numbers and types of ♂ offspring of each of the four different classes of ♀ ♀ listed below.

4, 6	24	11	18	16	42	42	19	23
5, 6	2	2	1	4	10	17	4	9
1, 2, 3	0	1	0	0	0	0	0	1
1, 2, 4	0	0	0	1	0	0	0	0
1, 2, 5	0	0	0	0	0	0	1	0
1, 3, 5	0	0	0	0	0	0	0	2
1, 4, 5	0	0	0	0	0	0	1	4
1, 4, 6	0	0	0	0	0	2	1	3
1, 5, 6	0	0	0	0	1	0	1	0
2, 3, 4	0	0	0	0	0	0	1	0
2, 4, 5	0	0	1	0	2	1	1	2
2, 4, 6	1	0	1	0	2	0	2	5
2, 5, 6	0	2	0	0	3	2	1	0
3, 4, 5	0	0	0	0	0	0	0	1
3, 4, 6	0	2	0	0	0	0	4	2
3, 5, 6	0	0	1	0	1	0	0	0
4, 5, 6	0	1	0	0	2	0	2	2
1, 2, 4, 6	0	0	0	0	0	1	0	0
2, 4, 5, 6	0	0	0	0	1	0	0	0
1, 3, 4, 5, 6	0	0	0	0	0	0	1	0
Totals	1975	1962	1407	1326	1929	1683	796	758
Grand totals	3937		2733		3612		1554	

SUMMARY

The effects of autosomal inversions on crossing over in the first chromosome were measured. Four different crosses were made: (1) controls (no inversions in the autosomes); (2) Curly inversions in the second chromosome, third chromosome normal; (3) Payne inversions in the third chromosome, no inversion in the second chromosome; (4) Curly inversions in the second chromosome and Payne inversions in the third chromosome simultaneously. In all four crosses crossing over was measured in the first chromosome by means of the alternated 7ple gene complex.

Autosomal inversions increase crossing over in the X chromosome. This increase is accompanied by a great increase in multiple crossing over. These results confirm those of SCHULTZ and REDFIELD on the negative correlation of the interchromosomal effect with the intrachromosomal effect of inversions on crossing over.

The Curly inversions have their greatest effect on the yellow end of the X chromosome. This effect decreases rapidly until the *cv-ct*^{6} region is reached and thereafter it remains constant. The Payne inversions have an approximately uniform effect on crossing over throughout the portion of the X chromosome marked in this experiment.

The combined inversions have an effect similar to that of the Curly inversions, that is, greatest in the *y-ac* region, and constant to the right of the *cv-ct*^{6} region. This effect is greater in all regions than that of either the Curly or Payne inversions when used singly.

The total increase in map length of the *y-f* interval caused by the inversions was greatest in the cross with the inversions combined and least when the Curly inversions were used alone.

Analysis of the strand data shows that while there was a decrease in non-crossover strands and an increase in multiple crossover strands; the single crossover strands, with the probable exception of those in the cross involving the Cy chromosome alone, remained constant. Here again the order of magnitude of the effects of the inversions on the strand data was Cy; Payne > Payne > Cy.

Tetrad analysis showed that there was a reduction in non-crossover and single crossover tetrads and an increase in multiple crossover tetrads. As in the above two cases the order of magnitude of the effects of the inversions was Curly; Payne > Payne > Curly.

The author wishes to acknowledge his indebtedness to Professors L. C. DUNN and D. E. LANCEFIELD and to Mrs. JEANNE C. MOSSIGE for their many helpful suggestions and to Mr. JACK GODRICH for preparation of figure 1.

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SEVENTH INTERNATIONAL CONGRESS OF GENETICS, AUGUST, 1937, USSR

The Seventh International Congress of Genetics will be held in the USSR in 1937. It is planned to have the sessions in Moscow, in the second half of August. Organization Committee: President, A. I. Muralov (President of the Lenin Academy of Agricultural Sciences); Vice-Presidents, N. I. Vavilov and V. L. Komarov; General Secretary, S. G. Levit; and members, N. P. Gorbunov, G. D. Karpechenko, B. A. Keller, N. K. Koltzoff, T. D. Lysenko, G. K. Meister, H. J. Muller, M. S. Navashin, and A. S. Serebrovsky. All scientists working in the field of genetics are invited to present their contributions. The titles and abstracts should reach the Organization Committee before February 15th, 1937. Detailed informations concerning the program, membership, exhibits, lodging and transportation are being prepared. Excursions to various parts of the USSR are on the schedule. Suggestions concerning the program or other features of the Congress will be appreciated.

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